Dr. Dr. Peter Fahrenkrug
Pinneberger Straße 43
25451 Quickborn
Germany

I, the undersigned,

Dr. Dr. Peter Fahrenkrug,

Pinneberger Straße 43, 25451 Qickborn, Germany,

have, at the request of Bayer HealthCare AG, Animal Health Division, 51368 Leverkusen, Germany,

reviewed the following documents

1. Limet, A (2003): Exploratory study on the activity of Bay 14-1877 tablets after oral administration on sub-gingival flora in cases of canine periodontal disease. Bayer Animal Health Report ID 27430.

2. Dellac, B (2003): Addendum to the final study report 142.089 and 142.090 entitled: Exploratory study on the activity of Bay 14-1877 tablets after oral administration on sub-gingival flora in cases of canine periodontal disease. Bayer Animal Health Report ID 27963.

3. Liège, P (2004): Evaluation of the efficacy and safety of BAY 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions.
   Bayer Animal Health Report ID 27428.

I declare that I have not been involved in the conduct of the exploratory study (ID 27430, ID 27963). I have been involved in the clinical field study (ID 27428) as fully blinded investigator and, thus, did not have any influence on the evaluation of study results. I further declare that I have not been involved in the preparation of the final study report.

Date: 6/6/05

Signature: Dr. Dr. P. Fahrenkrug Pinneberger Str. 43 Tel. 04106 - 6703 25451 QUICKBORN

Table of contents

Introduction

Periodontal disease is the most important cause for an early loss of teeth in dogs. The great numbers of toothless Yorkshire Terriers, Poodles, Chihuahuas – just to name a few affected breeds – can be attributed to periodontal disease. Unlike other bacterial infections, periodontal disease is caused by the accumulation and changes of a complex bacterial flora, which is referred to as dental plaque. If plaque is not constantly removed, gingivitis and periodontal disease will develop. During active phases, periodontal disease progresses by complex interactions between bacterial and host defence factors. The ultimate result will be the loss of the affected teeth. For a better understanding, important characteristics and processes of periodontal disease will be summarised before discussing the more specific aforementioned study reports.

Periodontal disease

Periodontal disease can be described in one sentence: Bacteria (plaque) accumulate on the surface of the teeth, gingival inflammation results, tissue destruction often follows if plaque accumulation is allowed to continue, and local and distant effects can be clinically significant (Harvey 1998).

Periodontal disease may occur in only a few teeth, in several teeth at a time or involve only one root of a multi-root tooth (Colmery and Frost 1986). Hence, it is more accurate to state that a particular tooth has periodontal disease rather than that a dog has periodontal disease (Harvey 1998). Any one dog can have one or more stages of periodontal disease in its mouth (Colmery and Frost 1986).

Prevalence of periodontal disease

Periodontal disease is probably the most common disease in dogs and almost all dogs over five years of age are affected by periodontitis (Harvey 1998). The overall prevalence of periodontal disease has been investigated by several authors and reported as 53% of 63 dogs (Golden *et al.* 1982), 57% of 1350 dogs (Harvey *et al.* 1994), 64% of 132 dogs (Hamp *et al.* 1984), 74% of 600 dogs (Bell 1967) and 97% of 62 dogs (Gad 1968). The frequency and severity of periodontal disease increases significantly with increasing age (Gad 1968, Hamp *et al.* 1984, Harvey *et al.* 1994) and decreases significantly with increasing body weight (Hamp *et al.* 1984, Harvey *et al.* 1994).

Predisposing factors

Malocclusion provides protected areas for accumulation of food debris and plaque (Harvey 1998) and constantly causes damage to the dentition and the periodontium by abnormal forces exerted by opposing teeth (Colmery and Frost 1986, Harvey 1998). Similar damage can be done by behavioural abnormalities such as fence, rock and bone chewing in bored dogs (Colmery and Frost 1986). Developmental defects such as retention of deciduous teeth also leads to increased accumulation of food and dental plaque (Colmery and Frost 1986). Enamel hypoplasia as an effect of febrile diseases in young growing dogs exposes porous dentinal layers to the oral environment and favours plaque and calculus accumulation (Colmery and Frost 1986). Mouth breathing in brachycephalic dogs has been postulated as a cause of gingivitis in dogs (Colmery and Frost 1986), but its role in the development of periodontal disease is disputed (Harvey 1998). An important predisposing factor for periodontal disease is the diet. Periodontitis can be considered a disease of civilisation as a natural diet keeps the amount of plaque below the threshold required to induce periodontitis (Harvey 1998). Harvey *et al.* (1996) found progressively less accumulation of calculus, less gingival inflammation and less periodontal bone loss in dogs that had access to various types of chewing material. Most effective were rawhide chews. Dry diets and chewing materials with abrasive action on the teeth are considered to retard plaque accumulation and development of periodontal disease (Harvey 1998). Periodontal disease may also have a hereditary component in some breeds such as Maltese and miniature Schnauzer dogs (Harvey 1998).

Tissues and structures involved

The tissues and structures affected by periodontal disease are the tooth, which is ultimately lost as a consequence of periodontal disease and the periodontium. The tissues surrounding each tooth are called the periodontium (*peri* = "around" and *odontos* = "tooth").

The hard tissues of the tooth include enamel, dentine and cementum, which is usually defined as part of the periodontium (Hennet 1995a). The enamel is the hardest substance in the body, covers the crown of the tooth and serves to grind and tear food. At its base, the crown has a bulge, the dental or enamel bulge. The roots of the tooth are covered by the cementum, the cementoenamel junction being the border between the crown and the root. The greatest (inner) part of the crown and the roots is formed by the dentine. The dentine is characterised by numerous dentinal tubules, which extend throughout the complete width and length of this part of the tooth. As discussed below, dentinal tubules can play a certain role in periodontal disease.

The periodontium consists of gingiva, periodontal ligament, root cementum and alveolar bone. It constitutes the attachment tissue of the tooth (Hennet 1995a) and is a well-designed system to hold the teeth in place in the jaws including a shock absorbing suspension system, which is primarily made up of the fibres of the periodontal ligament (Harvey 1998).

The gingiva is comprised of dense fibrous connective tissue, rich in collagen, which is covered by an outer layer of squamous epithelium. The gingiva forms a protective cover for both the alveolar bone and periodontal ligament. The free gingival margin has a fine tapered point that fits closely against the enamel surface. The free gingival margin also forms the gingival sulcus, which surrounds the teeth and can be up to 3 mm deep in dogs. From the bottom of the gingival sulcus to the cementoenamel junction, the junctional epithelium adheres via a hemidesmosomal attachment to the enamel surfaces. This hemidesmosomal attachment keeps the protective gingiva in close contact to the tooth, which itself protects the gingiva from injuries during chewing by the dental bulge.

The cementum is an avascular bone-like tissue covering the roots of the tooth. Deposition of cementum continues throughout life. It is a very important structure involved in tooth support being capable of both resorptive and reparative processes (Hennet 1995a).

The periodontal ligament consists of bundles of collagen fibres (Sharpey's fibres) that insert into tooth root cementum and alveolar bone and extend from the cementoenamel junction to the apex of the tooth. At the cementoenamel junction, collagen fibres of periodontal ligament and gingiva are interconnected. The periodontal ligament anchors the tooth to the alveolar bone, but also provides a flexible suspension system, which absorbs biting forces and spreads them to the surrounding bone. Would the teeth be rigidly fixed to the alveolar bone, they might crack during hard biting or chewing activity. The periodontal ligament space, i.e. the space between alveolar bone and tooth, is approximately 0.25 mm wide (Hennet 1995a) and contains many blood vessels and various types of cells, e.g. osteoclasts and osteoblasts or inflammatory cells in periodontal disease.

The alveolar bone supports the teeth during their biting action. The roots are tightly enclosed in the alveolar sockets, where they are flexibly fixed by the periodontal ligament. The interdental crest of the alveolar bone usually extends until approximately 1 mm apical of the cementoenamel junction (Hennet 1995a). Alveolar bone responds readily to external or systemic stimuli by resorption or apposition.

Pathogenesis of periodontal disease

As mentioned above, periodontal disease is caused by the accumulation of dental plaque. For dogs, this could be demonstrated by Lindhe *et al.* (1975). Within two weeks after cessation of oral hygiene, large amounts of plaque accumulated on the teeth of ten beagle dogs. Calculus was generally present after one month and gingivitis was obvious at that time. After two years, the dogs had developed periodontal disease characterised by loss of attachment and bone loss.

The development of dental plaque starts with the formation of the dental pellicle. This acellular film consists of salivary and nutritional glycoproteins, polypeptides and lipids and builds up on the tooth surfaces within seconds of exposure of clean teeth to saliva (Harvey 1998). The next step is the initial adherence of Gram-positive cocci and rods to the dental pellicle. These bacterial organisms colonised the dental pellicle and prepare the plaque surface for adherence of further bacteria like Gram-negative rods. Mature plaque is a rich mixture of Gram-positive and Gram-negative cocci and rods. It can develop within a few days and reaches its maximal thickness after ten days without oral hygiene (Colmery and Frost 1986). As the plaque thickens, the oxygen tension is greatly reduced in the innermost layers of the biofilm and anaerobes start to grow.

Inorganic substances from saliva, such as calcium compounds, are deposited into bacterial plaque, which is transformed into dental calculus (or tartar) by this mineralisation. Calculus can be observed within three days after mechanical cleaning of the teeth (Harvey 1998). The rough surfaces of calculus provide a platform for the attachment of additional dental plaque. Unlike plaque, calculus is not easy to remove from the teeth and its removal requires professional equipment and general anaesthesia.

The consequence of the accumulation of supragingival plaque and/or calculus is the development of gingivitis. Metabolic products of the bacteria and, later also the bacteria, enter gingival tissues and initiate the inflammation (Colmery and Frost, 1986; Harvey 1998). Gingivitis is a classical inflammation associated with tissue redness, oedema, bleeding, tenderness and impairment of the physiological function of the gingiva, jeopardising the self-cleaning mechanisms of the gingival sulcus provided by the saliva and gingival crevicular fluid. Swelling of the free gingival margin enlarges the gingival sulcus, providing additional space for the accumulation of subgingival plaque and calculus. However, at this stage, the junctional epithelium remains attached to the tooth surface and loss of periodontal attachment is not observed. The process is still reversible (Hennet 1995b).

If subgingival plaque is further allowed to accumulate, the inflammation becomes more severe. Vascular permeability is increased and large numbers of neutrophil granulocytes migrate through the sulcular and junctional epithelium as a response to the plaque bacteria (Harvey 1998). Monocytes are attracted to the site of inflammation and develop into macrophages (Hennet 1995b). Osteoclastic stem cells are similarly attracted to the area and start to resorb bone (Harvey 1998). Over time, the inflammatory response takes on a more chronic appearance and plasma cells and lymphocytes become more numerous (Harvey 1998). The inflammation results in loss of the hemidesmosomal attachment of the gingiva to the tooth and ongoing tissue destruction. True periodontal disease develops, which is characterised by apical migration of epithelial attachment and bone loss. The physiological gingival sulcus transforms into a pathological periodontal pocket. At this stage the damage is irreversible, but can usually be arrested and controlled with suitable treatment (Hennet 1995b, Nielsen *et al.* 2000).

The tissue destruction is caused by a complex interaction between direct microbial factors and indirect host defence factors, by which the connective tissues are largely destroyed by autodegradation (Harvey 1998). Microbial products directly harmful to the host tissues include enzymes, toxins and metabolites. Leukotoxins destroy neutrophils, whereas endotoxins (lipopolysaccharides, LPS) of Gram-negative bacteria like *Porphyromonas gingivalis* exert direct cytotoxic effects, effects on immune cells and stimulate bone resorption by osteoclasts. Enzymes like collagenase and acid phosphatase contribute to collagen degradation and bone resorption. Metabolic products such as ammonia, indole, short-chained fatty acids and volatile sulphur compounds are directly cytotoxic, inhibit collagen synthesis or are toxic to neutrophils. Cells of the host defence contribute to the destructive process. Neutrophils burst during ingestion of plaque bacteria or are destroyed by bacterial toxins, which leads to the release of further destructive enzymes like collagenase. Macrophages are stimulated by LPS to release cytokines like prostaglandin E_2, interleukins and tumour necrosis factor (DeBowes 1998), all of which mediate the inflammation and contribute to bone destruction.

If left untreated, the destructive process will lead to exposure of the root cementum and alveolar crest to the oral environment. Further bone loss will result in exfoliation of the affected tooth and, finally, to the resolution of the inflammation. The process is complete when new gingiva has grown over the now empty alveolus. Loss of the tooth can be seen as the ultimate host defence as the body is better off without the tooth rather than continuing to fight an ongoing infection (Harvey 1998).

Microbiology of periodontal disease

The initial colonisation of the dental pellicle is mainly done by *Streptococcus* spp. and *Actinomyces* spp. (Hennet 1995b). These bacteria synthesise extracellular polysaccharides, to which further bacteria, e.g. staphylococci, coliforms, lactobacilli, and many other species, are able to adhere. With extension of the supragingival plaque into the gingival sulcus, aerobes consume the available oxygen, thereby creating a low redox potential particularly at the bottom of the gingival sulcus. These environmental conditions favour the growth of anaerobic organisms. As the disease progresses, deeper periodontal pockets develop with heavy accumulation of bacteria that further lower the oxygen levels. Anaerobes take over and constitute approximately 95% of the subgingival flora in periodontitis (Hennet 1995b).

During this development, there is also a shift from the predominantly non-motile Gram-positive flora found in the supragingival plaque and the healthy gingival sulcus to a flora of Gram-negative motile anaerobic rods found in periodontal pockets (Eisenberg *et al.* 1991). As a general rule, one can say, that in periodontal health the flora is composed of 85% Gram-positive and 15% Gram-negative bacteria, whereas in periodontal disease this ratio is reversed to 80% Gram-negatives and 20% Gram-positives. This change can occur within two weeks when plaque is allowed to accumulate (Colmery and Frost 1986). Harvey *et al.* (1995) studied this period of transition, in that they took subgingival plaque samples from dogs with severe gingivitis that had not developed periodontitis around the sampled teeth. They found 41% Gram-positive bacteria and 59% Gram-negative bacteria, showing that the shift from Gram-positive to Gram-negative flora is well on its way in severe gingivitis. The shift of the bacterial flora in periodontitis could also be demonstrated by Isogai *et al.*

(1988) for dogs and is a well known fact in human dentistry. Hence, the isolation of mainly Gram-negative rods from dental pockets can be viewed as an indicator of periodontal disease.

It is known from human dentistry, that certain periodontal pathogens like *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and spirochetes are able to invade periodontal tissues, where they contribute to the destructive inflammatory process. Similar invasiveness of periodontal pathogens is also assumed in dogs (Sarkiala and Harvey 1993, Hennet 1995b, Nieves *et al.* 1997).

Periodontal disease is caused by plaque, which is a highly complex bacterial flora. Harvey *et al.* (1995), in their detailed investigations of the bacterial flora of subgingival plaque, identified as many as 60 different aerobic and anaerobic bacterial species or groups. Many of the plaque bacteria can initiate gingivitis if they are present in high numbers and it is this complexity of the bacterial plaque that has lead to the assumption that periodontal disease is caused by an overwhelming unspecific bacterial load in the gingival sulcus, the so called *non-specific plaque hypothesis*. Indeed, the bacterial volume and mix required to produce disease probably varies greatly from animal to animal and perhaps from site to site in the animal (Harvey 1998).

More recently an increasing amount of information has become available that periodontal disease is caused by a more specific bacterial flora, which resulted in the *specific plaque hypothesis*. The general conclusion is that tissue-destructive effects do not develop until Gram-negative anaerobic rods are present in large numbers and that black-pigmented anaerobic bacilli (BPAB) are the main offenders (Harvey 1998). In humans, the BPAB *Porphyromonas gingivalis* and *Prevotella intermedia* are now firmly implicated as periodontal pathogens, and there is an increasing amount of evidence that *Porphyromonas* spp., particularly *Porphyromonas gingivalis*, are implicated in canine periodontal disease (Harvey 1998). It should be noted here that the canine biotype of *Porphyromonas gingivalis* should now be referred to as a different new species *Porphyromonas gulae* (Fournier *et al.* 2001). Further new *Porphyromonas* spp. have only been isolated from dogs and cats so far. These are *Porphyromonas* (*P.*) *canoris*, *P. salivosa*, *P. cangingivalis*, *P. cansulci*, *P. crevioricanis* and *P. gingivicanis* (Harvey 1998). In the study of Harvey *et al.* (1995), *Porphyromonas* spp. and *Prevotella* spp. were the most frequently isolated anaerobes from subgingival plaque of dogs. Further anaerobes isolated from dogs were *Peptostreptococcus* spp., *Clostridium* spp., *Eubacterium* spp., *Propionibacterium* spp., *Fusobacterium* spp., *Bacteroides* spp., *Veillonella* spp., *Mobiluncus* spp. and many unidentifiable Gram-negative anaerobic rods (Harvey *et al.* 1995, Hennet 1995b). This shows the great complexity of the anaerobic bacterial flora in periodontal disease.

It has long been known that spirochetes also make up a high proportion of bacteria in plaque from sites with periodontitis. However, as spirochetes are extremely difficult to culture (Hennet and Harvey 1991, Harvey 1998), the knowledge on the role of these bacteria in periodontal disease is scarce. *Treponema denticola* and *Treponema socranskii* have been detected in plaque samples from dogs with the aid of monoclonal antibodies (Riviere *et al.* 1996). They were present in higher proportion in deep periodontal pockets, suggesting an involvement in periodontal disease.

However, the specific plaque hypothesis does not mean that we are dealing with a classical bacterial infection. The interactions between *Porphyromonas* spp., spirochetes and other anaerobic species are probably still very complex and none of the bacterial species would induce periodontal disease on its own. Indeed, Koch's postulate that a specific organism is isolated from disease, reproduces disease in healthy animals (the healthy gingival sulcus in this case) and can be re-isolated from the induced disease, cannot be proven for periodontal disease. The reason is that the site of disease is an external surface (the crown and later the root of the tooth), which is constantly exposed to a great variety of bacterial species (Harvey 1998). Hence, cultures from periodontal pockets routinely result in multiple and inconsistent isolations.

Systemic effects of periodontal disease

During the severe inflammation that characterises periodontal disease, bacteria gain access to gingival tissues and are found in close proximity to capillaries. Due to increased vascular permeability some bacteria can enter the bloodstream and cause bacteraemia. Such spontaneous bacteraemia has been detected in 15% of the dogs examined by Black *et al.* (1980). This observation can indeed be interpreted as a confirmation that certain bacteria are able to invade or at least colonise gingival tissues of dogs in periodontal disease.

It could be demonstrated both for humans and dogs, that routine dental procedures such as ultrasonic or mechanical dental scaling induce bacteraemia in a high percentage of patients. In humans, 70% of patients showed bacteraemia after dental scaling (Heimdahl *et al.* 1990). This rate was similar to the 67% found by Black *et al.* (1980) subsequent to ultrasonic scaling in dogs. The percentage of positive blood samples was correlated to the severity of periodontal disease, increasing from 57% in dogs with mild to 75% in animals with severe periodontitis (Black *et al.* 1980). In a more recent study, all of 20 dogs showed bacteraemia after ultrasonic scaling (Nieves *et al.* 1997). Seventeen dogs were bacteraemic within 20 minutes of the start of the dental procedure. Duration of bacteraemia was only marginally shorter than the duration of the dental procedure and ranged from 10 to 60 minutes. Ninety percent of dogs had Gram-negative bacteria cultured from their blood, whereas Gram-positive bacteria were isolated from 80% and anaerobes from 55% of the dogs (Nieves *et al.* 1997).

Commonly listed potential consequences associated with dental bacteraemia include endocarditis, glomerulonephritis, polyarthritis, polyvasculitis, auto-immune disorders, discospondylitis, endotoxaemia, organ abscesses and chronic pulmonary disease (Hennet 1995b). The most frequent causative organisms of endocarditis in dogs are *Streptococcus* spp. and *Staphylococcus aureus.* (Marcella 1998). These bacteria have been isolated both from subgingival plaque of dogs with periodontal disease (Harvey *et al.* 1995, Nieves *et al.* 1997) and from bacteraemia induced by dental scaling (Black *et al.* 1980, Nieves *et al.* 1997). Bacteraemia subsequent to dental manipulation may be a fundamental event in the cause of bacterial endocarditis (Black *et al.* 1980). Very recently, Tou *et al.* (2005) reported the first case of bacterial endocarditis in a dog that was directly linked to dental prophylaxis. Cardiopulmonary disorders of the geriatric dog may result from repeated low-grade infection by Gram-negative rods occupying periodontal spaces (Hamlin 1990). One scenario for the

development of pulmonary fibrosis and bronchitis might be that periodontal bacteria seed the bronchopulmonary tree via bacteraemia, attract neutrophils and macrophages, which release toxic enzymes resulting in chronic degenerative changes of the tissues (Hamlin 1990). Bacteria found in periodontal pockets such as *Actinobacillus* spp. and *Bacteroides* spp. are also recovered from the small airways of the lungs and thickened mitral valves (Hamlin 1990). In humans, *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*, all important periodontal pathogens, have been isolated from atheromas (Loesche 1999), underlining the potential significance of dissemination of dental anaerobic bacteria via the bloodstream. Anaerobes are involved in many infectious diseases of small animals, e.g. peritonitis, bone, joint and respiratory infections. A possible route of such infections is bacteraemia (Black *et al.* 1980). Animals with low-grade periodontal infection may present with episodes of hepatitis, kidney disease or gastrointestinal disease from the periodic bacteraemia that can occur during eating (Colmery and Frost 1986).

As described above, the majority of periodontal pathogens are Gram-negatives. These bacteria release endotoxins (LPS), which in turn mediate the local release of inflammatory cytokines. Activated macrophages produce a host of cytokines and other inflammatory mediators including lipid metabolites (prostaglandin E_2, tromboxane A_2, platelet activating factor), proteins (tumour necrosis factor, interleukins), enzymes and reactive oxygen intermediates (DeBowes 1998). In addition to local destructive effects and bacteraemia, periodontal disease may result in a systemic challenge with LPS and cytokines (DeBowes 1998). Cytokines can exert an endocrine effect on distant organs or tissues (DeBowes 1998).

In humans, untreated periodontal disease has been identified as a risk factor for atherosclerosis, thrombus formation, coronary heart disease, acute myocardial infarction, cerebrovascular accidents (stroke) and pre-term delivery of low birth weight babies (Loesche 1999). The correlation between periodontal disease and the aforementioned systemic diseases is considered to be due to the release of LPS, heat-shock proteins and cytokines into the bloodstream by Gram-negative periodontal pathogens (Loesche 1999). The risk is correlated with severity of periodontal disease. Pre-term birth of low weight infants is attributed to elevated levels of prostaglandin E_2, that eventually exceeds the threshold for induction of physiological contractions of the uterus (Loesche 1999). LPS and cytokines are known to promote atherosclerosis and thrombus formation and periodontal patients have elevated leukocyte counts and increased serum levels of fibrinogen and C-reactive protein, all of which are risk factors for cardiovascular disease (Loesche 1999). Periodontal pathogens like *Porphyromonas gingivalis* and *Prevotella intermedia* have been detected in atheromatous plaques, the presence of *P. gingivalis* being explained by expression of a platelet aggregation factor (Loesche 1999). Another link between poor oral hygiene and cardiovascular disease could be a cross-reaction of antibodies directed against bacterial heat-shock proteins (hsps) with hsps of the vascular endothelium. This auto-immune reaction damages the vascular endothelium and contributes to development of atherosclerosis (Loesche 1999).

In a detailed histopathological study in 45 dogs, increasing severity of periodontal disease was significantly correlated with myocardial degeneration and morphological alterations of kidney (glomerula and interstitium) and liver parenchyma (DeBowes *et al.* 1996). This study clearly supports the hypothesis that periodontal disease in dogs can have systemic effects on other organs.

Clinical signs of periodontal disease

As plaque accumulates on the teeth, the first clinical sign is increased halitosis (foetor ex ore) caused by volatile sulphur compounds produced by plaque bacteria (Colmery and Frost 1986). Plaque becomes visible as whitish soft deposits on the surfaces of the crown, on the gingival margin and in the interdental spaces. As the plaque becomes mineralised, calculus deposits can be seen supragingivally as a thin build-up of yellow-brown material at the gingival margin (Colmery and Frost 1986). Subgingival calculus is usually harder and dark brown in colour (Colmery and Frost 1986).

In gingivitis, the initially coral pink and firm marginal gingiva becomes swollen, oedematous and friable in some cases (Harvey 1998). Reddening and rounding of the free gingival margin is observed (Colmery and Frost 1986). With increasing severity of gingivitis, there will be spontaneous gingival bleeding or bleeding may occur as a result of exploring the pocket with a periodontal probe (Harvey 1998). In some dogs with gingivitis, gingival hyperplasia has been described, e.g. for Boxer dogs and other medium-size and large breeds of dogs (Harvey 1998). In these animals, the gingival margin becomes cauliflower-like (Colmery and Frost 1986). A clear line of demarcation between diseased and normal tissue is visible in dogs with gingivitis (Colmery and Frost 1986).

As gingival inflammation and tissue resorption continues, attachment of the sulcular epithelium and then the periodontal ligament to the enamel and cementum is lost. The physiological gingival sulcus gradually widens and deepens to become a pathological periodontal pocket. Periodontal pocket depth can be measured with a periodontal probe. In healthy dogs, the gingival sulcus is not deeper than 3 mm. In early periodontitis, pockets are up to 4 mm, in moderate periodontitis up to 6 mm and in severe periodontitis more than 6-7 mm deep (Nielsen *et al.* 2000). In progressing periodontal disease, loss of attachment is often characterised by apical recession of the gingival margin. Root cementum becomes visible between the cemento-enamel junction and the free gingival margin and exposed to the oral environment. With further recession and destruction of the gingiva and periodontal ligament, the alveolar crest becomes involved and bone is resorbed. Radiographs will show an increased erosion of the alveolar process with a loss of bone height and density (Colmery and Frost 1986).

As bone is lost between the roots of multi-rooted teeth, a periodontal probe may be inserted into the gap formed by the crown of the tooth, the roots and the remaining periodontium, or even completely passed from buccal to lingual or palatal beneath the crown. This is called partial or complete furcation involvement or a furcation defect (Harvey 1998). This furcation defect is functionally significant as it provides a secluded space for accumulation of plaque and food debris, which cannot be

removed by standard oral hygiene measures such as tooth brushing (Harvey 1998). Pus is also frequently present around the roots of the teeth (Colmery and Frost 1986). With further bone resorption, an intrabony pocket is formed, which can reach a depth of 15 mm on the palatal side of a canine tooth of large breeds (Harvey 1998). Sometimes, closed infections develop deep inside a periodontal pocket that lead to abscessation and fistulation (Harvey 1998).

Further root structure is separated from its attachment and tooth mobility develops (Colmery and Frost 1986, Harvey 1998). This increases the leverage forces acting on the tooth, which compress the blood supply and stimulate further bone resorption (Harvey 1998). Mobility becomes more and more severe and in the end the tooth may wiggle when gently touched (Harvey 1998). Sometimes, the teeth are only held in place by massive deposits of calculus. Ultimately, the teeth will be lost.

Apart from these direct oral signs of gingivitis and periodontal disease, the animal shows severe halitosis, difficulty of eating, anorexia, pawing at the mouth and bleeding from the mouth (Colmery and Frost 1986). General depression up to lethargy, pyrexia and aggressive unpredictable behaviour are also observed (Harvey 1998). The mouth may also be painful due to ulceration of the buccal mucosa opposite sites of calculus accumulation. A further sign may be drooling, i.e. increased salivation.

Treatment of periodontal disease

In 27 years as a human dentist and specialised veterinary dentist, I have not only treated more than 5000 human patients, but also nearly 2000 dogs and 400 cats against periodontal disease. Hence, besides referring to published literature on treatment of periodontal disease, this chapter also reflects my personal experience.

Periodontal disease is caused by plaque. Allow plaque to accumulate and disease will result. Prevent accumulation of plaque and disease will never develop. Indeed, Lindhe *et al.* (1975) could completely prevent gingivitis and periodontal disease by twice daily meticulous tooth brushing in each of ten beagle dogs over a period of four years. However, without such heroic measures, complete prevention of disease is impossible (Harvey 1998).

Notwithstanding the above, the main goal in periodontal therapy is prevention of plaque accumulation and removal of already accumulated plaque and calculus. This is best achieved by professional mechanical periodontal therapy, which is performed under general anaesthesia. The animal should be intubated to avoid aspiration of debris and bacteria. Mechanical periodontal therapy consists of supra- and subgingival scaling, root planing, tooth polishing, sulcular lavage and, sometimes, periodontal surgery (Gorrel and Robinson 1995). In the following, these techniques will only be briefly described. Detailed information can be found in the comprehensive paper by Gorrel and Robinson (1995). In general, teeth with poor prognosis, e.g. extensive mobility, should be extracted in order to faster resolve the pathological processes around these teeth.

Plaque on the crowns of the teeth is removed by supragingival scaling. Sickle-shaped mechanical scalers are gently pulled from the gingival margin to the tip of the crown. Sonic or ultrasonic scalers are often used as they provide fast and accurate cleaning. However, care must be taken that these instruments are only used for a few seconds per tooth at a time in order to avoid damage of the pulpa by frictional heat. Calculus deposits can be removed with special calculus pliers or calculus forceps.

Subgingival scaling removes subgingival plaque and calculus. Root planing creates an even surface of the root cementum thereby favouring regain of attachment and decreasing the re-accumulation of plaque. Furthermore, toxins that have entered the superficial layers of the cementum are removed. Plaque and calculus removal and root planing can be performed at the same time with a subgingival curette. Sonic or ultrasonic scalers can be used as long as the cooling water supply inside the periodontal pocket is sufficient to avoid heat damage of the tissues. It may be necessary to gain access to deep pockets via a gingival flap. Gingiva is cut with a scalpel, lifted from the periosteum and then carefully held aside to be able to clean the complete root surfaces. Finally, the gingiva is sutured back into position. These access flaps can only be used in dogs with good owner compliance regarding dental home care.

Sulcular lavage is important after subgingival scaling as it will remove remaining loose plaque, calculus pieces and other debris. Usually, a blunt syringe or lacrimal catheter is used to flush the periodontal pockets with physiological saline solution or dilute chlorhexidine solution.

Every scaling produces minor scratches on the tooth thereby creating rough surfaces, which predispose to plaque accumulation (Gorrel and Robinson 1995). Polishing not only results in a smoother tooth surface, but also removes remaining plaque. A soft rubber cup on a low-speed hand piece and slightly abrasive prophylaxis pastes are used. The soft rubber cup forms a flared edge, by which subgingival areas are accessible to polishing. Again, care must be taken not to damage the dental pulpa by frictional heat. A study, in which I participated, showed that polishing after scaling results in significantly reduced re-accumulation of plaque and calculus, which will extend the intervals between mechanical periodontal therapies (Pollmeier 1994, Röcken *et al.* 1996).

Periodontal surgery, which is also referred to as gingivoplasty, is used to remove or reduce the depth of periodontal pockets. Gingivoplasty is often used in cases of gingival hyperplasia in order to re-create the normal gingival lining and sulcus depth around the teeth. This serves the prevention of plaque accumulation and development of periodontal disease in the pseudo-pockets formed by gingival hyperplasia. Reduction of periodontal pocket depth by surgery has the same purpose. For periodontal surgery to be successful, a high level of dental home care is required.

Professional periodontal therapy can only have sustained effects, if dental home care is performed by the owner. This daily removal of plaque is a prerequisite of prevention of disease development or progression. If no home care is instituted, plaque will rapidly re-accumulate after mechanical dental cleaning and periodontal disease will progress. Tooth brushing is by far the most effective way to remove plaque from the dentition. Special tooth pastes for dogs are available. Tooth brushing can be combined with plaque retardants such as chlorhexidine. This is probably the most effective home care method (Hennet 1995b). However, even with ideal home care, most animals will still need to have their teeth cleaned professionally at regular intervals (Gorrel and Robinson 1995). Dental home care depends on the co-operation of the animal, owner ability and owner compliance. Unfortunately, compliance with home care instructions is poor for many owners. A survey conducted in the United States revealed that six months or longer after periodontal treatment only 53% of the owners showed good compliance, whereas 38% were not brushing the teeth at all (Miller and Harvey 1994). Compliance with dental home care instructions is not better in Germany and Europe according to my personal experience. It should also be noted that chlorhexidine is not well tolerated by quite a few dogs due to its pungent taste.

The aforementioned elements of professional periodontal therapy in combination with dental home care are, and no doubt will be, the mainstay of the treatment of periodontal disease. Periodontal disease can be controlled in many, if not most, dogs by these measures. However, we observe great variations in the speed of re-accumulation of plaque and calculus between different breeds in daily practice. Response to mechanical periodontal therapy is worse in some dogs than in others and periodontal disease progresses in some dogs despite mechanical debridement has been performed. Taking into account the varying degree of compliance with dental home care instructions, it becomes clear that not all dogs can be treated mechanically alone. Some animals will need adjunctive therapy with broad-spectrum systemic (or topical) antimicrobials. The emphasis must be put on the word "adjunctive" as no animal should be treated against gingivitis or periodontitis with antibiotics alone. It should be further emphasised that only some animals will need adjunctive therapy and that it cannot be the goal of veterinary dentistry to treat every dog showing periodontal disease with antimicrobials.

Further valid general arguments for the use of antimicrobials in periodontal disease have been forwarded by Hennet (1995b): Administration of antibiotics (i) may decrease the bacterial load and prevent detrimental systemic infections, (ii) is necessary when signs of other systemic diseases are present, (iii) may enhance the success of dental treatments and (iv) may sometimes be indicated as salvage procedure if no other treatment can be performed.

The rationale for systemic antimicrobial use is that mechanical debridement may not adequately detoxify the periodontium and that the host immune system may not be capable of eradicating periodontal pathogens (Levin 1999). Mechanical treatment alone cannot remove invasive bacteria located in the gingiva and the periodontal ligament or bacteria residing in confined spaces such as the dentinal tubules and the alveolar bone (Sarkiala and Harvey 1993, Levin 1999). These bacteria constitute a reservoir for post-prophylaxis re-infection (Levin 1999).

Indications for the prudent use of antimicrobials in the adjunctive treatment of periodontal disease have been described in the literature and are discussed below.

1. Antimicrobial therapy directed against suspected periodontal pathogens can provide additional improvement in severe, refractory or recurrent cases of periodontitis when used in conjunction with dental prophylaxis (Sarkiala and Harvey 1993). All these cases would benefit from judicious use of antibiotics (Levin 1999). These are the dogs mentioned above, which are less responsive or non-responding to standard periodontal therapy followed by dental home care. Additional and more sustained improvements of periodontal condition could be shown for the combination of mechanical and adjunctive antimicrobial therapy in comparison to mechanical cleaning alone, both in humans (Gordon *et al.* 1990, Söder *et al.* 1990, Eisenberg *et al.* 1991, Loesche *et al.* 1992) and dogs (Heijl and Lindhe 1980, Sarkiala 1993, Nielsen *et al.* 2000). In humans, these beneficial additive effects have been mainly studied with clindamycin and metronidazole, in dogs with metronidazole (Heijl and Lindhe 1980), tinidazole (Sarkiala 1993) and clindamycin (Heijl and Lindhe 1980, Nielsen 2000). All the aforementioned and further studies (e.g. with a spiramycin-metronidazole combination) showed a reduced need for surgery, a more prolonged decrease of periodontal pathogens and/or an improved periodontal health as expressed by clinical indices (Hennet 1995b). In two studies, it could be demonstrated that antimicrobial therapy plus scaling induced a more sustained re-establishment of healthy periodontal flora (increase in Gram-positive, but decrease in Gram-negative bacteria) than scaling alone (Walker and Gordon 1990, Eisenberg *et al.* 1991). This beneficial effect on the periodontal flora lengthens the "rebound time" before periodontal disease becomes clinically obvious again when effective plaque control is not performed (Sarkiala and Harvey 1993).

2. The systemic effects of periodontal disease in humans and dogs have been described above. Based on this knowledge, patients with a high risk of systemic complications from bacteraemia would clearly benefit from systemic antimicrobial therapy (Levin 1999). Particularly at risk are immunocompromised animals, animals with organic or metabolic failure, with heart disease, prosthetic joints or other implants (Hennet 1995b). Antimicrobial therapy during dental prophylaxis or extractions is similarly recommended by Sarkiala and Harvey (1993) for dogs with cardiac disease, immunosuppressive diseases or receiving immunosuppressive therapy and for dogs with other systemic diseases causing clinical illness such as diabetes mellitus, hyperadrenocorticism and hepatic or renal disease. The prevention of bacteraemia in veterinary dentistry by administration of antimicrobials is also recommended by various other authors (Black *et al.* 1980, Colmery and Frost 1986, Marcella 1988, Hamlin 1990, Zetner and Thiemann 1993, Gorrel and Robinson 1995). The risks of dental bacteraemia have also led to the recommendation of antimicrobial endocarditis prophylaxis in humans undergoing periodontal surgery, scaling, root planing and probing, both in the original and updated guidelines of the American Heart Association (Dajani *et al.* 1990, Dajani *et al.* 1997). The fist dose should be given at least 24 hours prior to the procedure (Sarkiala and Harvey 1993). Although some authors favour the use of only a few doses of antimicrobials (Hennet 1995b, Dajani *et al.* 1997), it should be pointed out that it is prudent to use antimicrobials for a full treatment course also in this more prophylactic field in order to minimise the possibility of selection of resistant bacteria. The full treatment course according to the instructions in the

product literature will also be required to achieve the beneficial effects described in the previous paragraph.

3. Systemic administration of antimicrobials is also indicated pre-, peri- and postoperatively when periodontal therapy is conducted in conjunction with other surgery (Levin 1999), such as excision of subcutaneous masses like mammary tumours. Antibiotic therapy prevents seeding of distant surgical sites with organisms from the dental bacteraemia (Levin 1999). Direct contamination of wounds can occur in the mouth when healthy gingival tissue or bone is exposed to the oral environment during periodontal surgery (Hennet 1995b).

4. In humans, periodontal disease is often treated according to a long term treatment plan, which is also referred to as staged periodontal procedure (Levin 1999). However, such staged procedures are not possible in dogs as all of them would require general anaesthesia. In dogs with periodontitis and severe gingivitis, it is difficult to make a decision whether or not periodontal surgery may be required due to the extensive swelling of the gingiva. Therefore, antimicrobial therapy before mechanical periodontal treatment will greatly enhance the accuracy of clinical decisions during the subsequent procedure in such dogs (Levin 1999). Furthermore, it is a well known fact that healthy gingival tissues will heal much faster after periodontal surgery than oedematous inflamed gingiva. In general, periodontal surgery should not be conducted on severely inflamed gingiva, as this would greatly reduce accuracy and effectiveness of the surgical procedure. Antimicrobials are very useful to condition the periodontium for surgery.

5. Mechanical periodontal therapy is only tolerated by dogs under general anaesthesia, which always poses a certain risk to the animals. As discussed under point no. 1, adjunctive antimicrobial therapy can sustain a favourable treatment outcome. As dental home care is often not correctly performed (Miller and Harvey 1994), frequent re-evaluation and frequent treatments are necessary, each requiring general anaesthesia. Hence, an improvement of healing and an extension of the disease-free interval may be very helpful (Sarkiala and Harvey 1993, Hennet 1995b). The judicious use of adjunctive antimicrobial treatment will increase the length between professional periodontal treatments and thus reduce the number of anaesthetic episodes required during the lifetime of the individual animal (Sarkiala and Harvey 1993). Moreover, frequent mechanical procedures like scaling can damage the tooth and periodontium (Gorrel and Robinson 1995) and even be a cause for gingival recession and progression of periodontal disease. Hence, an extension of treatment-free intervals by antimicrobial therapy would also increase the recovery period of the periodontium after mechanical procedures.

6. If calculus deposits have led to ulceration of the buccal mucosa, this ulcerative stomatitis has to be treated with potent antimicrobial drugs along with mechanical removal of calculus and initiation of prevention of re-formation of calculus.

7. Ultrasonic scaling produces a fine aerosol containing large numbers of pathogenic and facultatively pathogenic bacteria (Sarkiala and Harvey 1993). Up to 1700 bacteria are aerosolised within 60 seconds and can be inhaled by the operators (Zetner and Thiemann 1993). A five day treatment course with clindamycin greatly reduced pollution of the environment with aerosolised bacteria, thus contributing to operator safety (Zetner and Thiemann 1993). I have myself suffered from chronic bronchitis before veterinary dentistry discovered this aerosolisation of bacteria and before we started to wear mouth and nose protection during dental scaling. Prudent use of antimicrobials in veterinary dentistry precludes their use for the sole purpose of reduction of bacterial air pollution. However, this reduction of air pollution is a significant additional benefit of antimicrobial therapy, whenever an animal has to be treated for one of the other indications listed above.

As discussed above, only those dogs with periodontal disease should be treated with antimicrobials, for which periodontal treatment goals cannot be achieved with mechanical procedures alone. The indications have also been clearly defined. However, there is no clear decision point, e.g. a certain severity or grade of periodontal disease, beyond which antimicrobial treatment must be started. It should be remembered that only one root of a multi-rooted tooth or almost all teeth can be affected by periodontal disease (Colmery and Frost 1986). Hence, the indication for the use of antimicrobials in periodontitis is not a question of number of teeth involved, but rather of the desired treatment outcome. If a functionally important tooth such as a canine or carnassial tooth has to be salvaged and this cannot be achieved by mechanical periodontal therapy, periodontal disease of only that one tooth would constitute an indication for the use of antimicrobial drugs.

Ideally, antimicrobial therapy should always be based on culture results and subsequent susceptibility testing. However, various problems are encountered with subgingival plaque samples. The major periodontal pathogens are black-pigmented Gram-negative anaerobic rods (Harvey 1998). These bacteria are difficult to culture and the statement by Hennet (1995b) does still apply that most human and veterinary microbiological laboratories are unfamiliar with such bacteria. Identification and susceptibility testing of anaerobes is not routinely performed (Hennet 1995b), with the exception of some research projects in specialised laboratories. Hence, most veterinary practitioners have to face the situation that no microbiological laboratory is available within a reasonable distance that offers anaerobic diagnostics. Furthermore, a pathological periodontal pocket is not one entity with an evenly deep, smooth bottom of the pocket all around the tooth. To the contrary, many secluded niches of varying shape and depth can be found in most periodontal pockets. Each of these niches can harbour a bacterial flora differing in composition from other niches within the same pocket. It has also been shown, that the microbial composition of subgingival plaque is different at different depths of the same periodontal pocket (Soames and Davies 1974). Hence, different culture results can be obtained depending on what niche or what depths of a pocket is actually sampled. Microbiological samples from periodontal pockets are usually taken with absorbent paper points, which are inserted into the pocket and left in place for a few seconds. Only bacteria that come in direct contact with the paper point will adhere and the culture will at best give a semi-quantitative overview of the bacterial flora present in the sampled niche. Hence, the isolation of certain periodontal pathogens does not tell

a lot about their number actually present in the periodontal pocket. Dilution of the sample by saliva or increased volumes of gingival crevicular fluid can decrease the numbers of bacteria isolated and even push some less frequent anaerobic species below the limit of detection. Blood absorbed by the paper point reduces the viability of anaerobes during transport and reduces bacterial isolation rates. The interpretability of anaerobic culture is further impaired by fact, that not all anaerobes are cultivable, as are not the spirochetes, either. Taking into the account the complexity of the subgingival bacterial flora (Harvey *et al.* 1995), it becomes obvious that it will never be certain if the bacteria obtained by anaerobic culture are the predominant or causative organisms. If periodontal pockets are sampled over time to monitor changes of the subgingival flora, sampling of exactly the same niche of the pocket will be difficult, if not impossible. Hence, monitoring of clear cut effects of mechanical or antimicrobial periodontal therapy is far more difficult than for classical bacterial mono-infections. This observation will also be of relevance when discussing the results of the exploratory study with pradofloxacin. In conclusion, although desirable, anaerobic culture and susceptibility testing of subgingival bacteria are of limited value only, but time consuming, expensive and treatment delaying. Thus, selection of the antimicrobial agents depends on the knowledge of the bacteria considered to be periodontal pathogens (Hennet 1995b), which are mainly the black-pigmented Gram-negative anaerobic bacilli (Harvey 1998). This means, that antimicrobials with proven activity against black-pigmented anaerobic bacilli (*Porphyromonas* spp., *Prevotella* spp.) have to be selected.

Harvey *et al.* (1995) isolated 60 different species or groups of anaerobic and aerobic bacteria from subgingival plaque of dogs. This alone demonstrates the complexity and diversity of the periodontal flora of dogs. Interestingly, a total of 160 out of 462 strains (35%) could not be identified. Among these strains were 83 Gram-negative anaerobic rods (18%), forty-six Gram-negative aerobic rods (10%), twenty-seven Gram-positive aerobic bacilli (6%) and four (1%) other strains (3 anaerobes, 1 aerobe). Hence, far more than 60 species will be contained in subgingival plaque samples, as it is known from human dentistry that up to 300 different bacteria have been isolated from periodontal pockets. The periodontal flora is a complex mixture of Gram-positive and Gram-negative anaerobic and aerobic rods and cocci. Antimicrobials to be used under such conditions have to be active against the anaerobic bacteria involved in periodontal disease and also against bacteria that are not considered periodontal pathogens, but are disseminated to distant organs via bacteraemia or involved in ulcerative stomatitis. Given all this, only broad-spectrum antimicrobial drugs can be expected to meet these requirements. It should be noted in this context, that treatment with penicillin, a small-spectrum beta-lactam antibiotic, was not able to prevent dental bacteraemia (Black *et al.* 1980). In human dentistry, certain forms of periodontal disease are more and more considered treatable anaerobic infections and systemic, broad-spectrum antimicrobials are recommended (Loesche 1999). As a general rule in the treatment of anaerobic infections, bactericidal antibiotics should be used (Sarkiala and Harvey 1993, Hennet 1995b).

Antimicrobial drugs have plaid and will play an important role in the adjunctive therapy of periodontal disease. However, only four products are currently registered for the use in oral infections and/or periodontal disease. These are

- The combination of metronidazole and spiramycin (Suanatem / Stomorgyl, Merial). The product is registered for the treatment of periodontal infections. Metronidazole is bactericidal and has a broad-spectrum of activity against Gram-negative and Gram-positive anaerobes, spiramycin extends the spectrum to mainly Gram-positive aerobes. Weaknesses of this combination are expected against Gram-negative aerobes.

- Clindamycin hydrochloride (Cleorobe / Antirobe, Pfizer, and generic products). Clindamycin is a broad-spectrum bacteriostatic antimicrobial, which is active against Gram-positive aerobes and obligate anaerobes. Again, Gram-negative aerobes are not susceptible to the drug. The product is registered for the treatment of dental infections and "to help provide antimicrobial cover during dental procedures".

- Amoxycillin/clavulanic acid (Synulox, Pfizer). This combination shows broad-spectrum activity against anaerobes, Gram-positive and also Gram-negative bacteria. However, activity against Gram-negative aerobic bacteria is reduced in comparison to the activity against Gram-positive bacteria. Only the 500 mg tablet is registered for the treatment of gingivitis.

- Doxycycline hyclate released from a biodegradable polymer, which is injected into the periodontal pockets (Doxirobe Gel, Pfizer). Doxycycline has broad-spectrum activity against many Gram-positive and Gram-negative aerobes and, in high concentrations as they are achieved with this product in the gingival sulcus, also against anaerobes. The product is registered for the treatment of periodontal infections. Doxirobe Gel acts via local release of doxycycline inside the periodontal pocket, so that efficacy against bacteria residing in periodontal tissues is limited and absent against bacteria entering the blood circulation during mechanical dental cleaning. Recent experience with this product will be published in the August, 2005, issue of "Der Praktische Tierarzt" by Zetner and Stoian, coinciding with the news that Doxirobe Gel has been taken from the market by the producer.

None of the quinolones has been registered for the treatment of periodontal disease. The reason is the lack of activity of the relatively old veterinary compounds against anaerobic bacteria. However, quinolones penetrate well into periodontal tissues and reach high concentrations in gingival crevicular fluid (Sarkiala and Harvey 1993). The activity of new generation quinolones against anaerobic bacteria has been discussed in the Microbiological Expert Report. It has been shown that new quinolones such as moxifloxacin and trovafloxacin possess excellent *in-vitro* activity against anaerobes. As detailed in the Microbiological Expert Report, pradofloxacin, which belongs to the same new generation of quinolones, has an activity against anaerobic bacteria that is at least three-fold increased compared to other veterinary quinolones.

As there are now only three products available for the treatment of periodontal disease (strictly speaking, only Stomorgyl and Antirobe are registered and remain available for this indication in EU Member States) and as increasing resistance rates to the older products may be encountered, alternative products would be most welcome. The suitable spectrum of activity as discussed by the Microbiological Expert and the clinical results discussed below, make pradofloxacin a promising alternative for adjunctive antimicrobial therapy of periodontal disease in dogs.

Studies with pradofloxacin

A. Exploratory study

The results of the exploratory study with pradofloxacin have been reported by

1. Limet, A (2003): Exploratory study on the activity of Bay 14-1877 tablets after oral administration on sub-gingival flora in cases of canine periodontal disease. Bayer Animal Health Report ID 27430.

and

2. Dellac, B (2003): Addendum to the final study report 142.089 and 142.090 entitled: Exploratory study on the activity of Bay 14-1877 tablets after oral administration on sub-gingival flora in cases of canine periodontal disease. Bayer Animal Health Report ID 27963.

Two groups of eight female beagle dogs with periodontal disease were included in the study at a privately owned breeding kennel. One group was treated with Stomorgyl (metronidazole + spiramycin) at dose of 12.5 mg/kg body weight (b.w.) metronidazole plus 75,000 i.U./kg b.w. spiramycin twice daily. The other group was treated with 3 mg/kg b.w. pradofloxacin once daily. Treatment duration was six consecutive days for both groups.

For each dog, the tooth with maximum loss of attachment was selected in each quadrant of the dentition. Hence four periodontal pockets (sites) were monitored per dog, i.e. a total of 64 sites. Dogs were allocated to the two groups based on initial loss of attachment to ensure that mean loss of attachment was similar between the groups. Both treatment groups were comparable before treatment initiation regarding the parameters age, body weight, loss of attachment and subgingival bacterial characteristics.

The clinical periodontal parameter assessed in this study was loss of attachment, which is a measure for the apical migration of epithelial attachment of gingiva and periodontal ligament during periodontal disease. Loss of attachment has to be measured from a defined reference position on the tooth in order to exclude effects of decreasing pocket depth by reduction of gingival swelling. Such fixed reference positions can be the cemento enamel junction or, more accurately, a permanent mark on the tooth surface. In this study, the reference point was a small hole that was drilled in the corona of the tooth and filled with amalgam. Loss of attachment was measured as the distance from this amalgam filling to the bottom of the pocket with a manual pressure sensitive periodontal probe. Microbiological parameters were

the development over the study period of the total subgingival flora count, the percentage of sites positive for periopathogens, the proportion of periopathogens in the total flora and the ratio of Gram-positive to Gram-negative organisms in subgingival plaque. All parameters were recorded on day -1, day 7, day 14 and day 28 of the study.

Both treatments significantly reduced loss of attachment over the study period. Thus, true regain of attachment was induced by both antimicrobial compounds. Considering the pathogenesis of periodontal disease discussed above, this is an important finding that demonstrates the beneficial properties of both compounds in the prevention of disease progression. Although the differences between the groups were not statistically significant, pradofloxacin treatment had a more pronounced effect on loss of attachment. The reduction in the pradofloxacin group was 0.47 mm compared to 0.32 mm in the Stomorgyl group.

The total subgingival bacterial count was significantly reduced by both treatments over the study period from values around 2×10^7 cfu/ml of sampling medium before treatment to approximately 5×10^5 on day 7 and 7×10^5 cfu/ml on day 28, i.e. 21 days after the end of treatment. Although bacterial counts increased close to pre-treatment values between day 7 and day 14, this effect can be considered beneficial, as the total bacterial load in the pockets was still significantly reduced three weeks after cessation of antimicrobial treatment. As we know from the pathogenesis of periodontal disease, a reduction of the subgingival bacterial count will result in reduced gingivitis. Significant differences between the treatment groups were not observed.

The periopathogens, which were statistically analysed in this study were *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*. On day 7, these bacteria had totally disappeared in both treatment groups. In the pradofloxacin group, they started to re-appear on day 14, in the Stomorgyl group on day 28. The proportion of periopathogens was significantly lower in the Stomorgyl group on day 14 and day 28 in comparison to the dogs treated with pradofloxacin. In addition, the percentage of sites positive for periopathogens was significantly lower in the Stomorgyl group on day 14. Although these statistical analyses are correct without any doubt, it has to be pointed out that the parameters used were not the most reliable ones. As discussed above in section 'treatment of periodontal disease', the numbers of periopathogens can only be determined semi-quantitatively. Furthermore, it is very difficult to sample exactly the same niche or depth of a periodontal pocket upon separate occasions, particularly after antimicrobial periodontal treatment that has led to changes of pocket depth via regain of attachment. Hence, the periopathogen counts of this study had a high degree of inherent inaccuracy and it is well possible that they were not representative of the actual microbiological situation in the periodontal pockets of the dogs. Keeping all this in mind, it should be emphasised that there was a statistically significant reduction of the proportion of periopathogens over the study period, both for Stomorgyl and pradofloxacin. The rate of periopathogens was <7% in the pradofloxacin group on day 28 of the study, which lies well within the proportion of periopathogens observed in periodontal health.

Looking at Appendix 3 to study report ID 27430, the tabulated individual microbiological raw data reveal that seven further periopathogens were sampled and counted in addition to *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*. These were *Eikenella corrodens*, *Capnocytophaga ochracea*, *Porphyromonas canoris*, *Porphyromonas cangingivalis*, *Porphyromonas cansulci*, *Fusobacterium nucleatum* and *Campylobacter rectus*, all of which have been reported as putative periodontal pathogens (Hennet 1995b, Harvey 1998). They were apparently considered less important by the statistician of this study and excluded from the statistical analysis. However, when the additional seven species are included in the analysis of sites positive for periopathogens, the results are quite different from those reported for the three periopathogens analysed by the statistician. On day -1 of the study, 56.3% of the sites were positive in the Stomorgyl and 59.4% in the pradofloxacin group. On day 7, there were still 56.3% of positive sites in the Stomorgyl group, but only 43.7% in the pradofloxacin treated groups. On day 14, positive sites were observed in 75% and 68.8% of the Stomorgyl and pradofloxacin treated animals, respectively. On day 28, the rate of positive sites was 68.8% for Stomorgyl and 73.1% for pradofloxacin. Thus, there was a slight advantage for pradofloxacin on days 7 and 14, but a slight advantage for Stomorgyl on day 28. Only few sampling sites were consistently negative or positive throughout the study. In the Stomorgyl group, 12 out of 18 sites positive on day -1 were still positive on day 7, but six previously negative sites became positive. On day 14, four sites positive on day 7 had become negative, whereas ten previously negative sites had turned positive. On day 28, seven sites positive on day 14 were now found to be negative, but five negative sites positive. The findings in the pradofloxacin group were very similar. The variability of the species of periopathogens identified in the positive samples was even greater than the predictability for a site to be positive or negative upon repeated sampling. These observations confirm that bacteriological sampling of periodontal pockets yields highly variable results, which should, therefore, be interpreted with caution. Hence, the reported disadvantage for pradofloxacin regarding the three periopathogens analysed might well have been due to sampling inaccuracy rather than lack of product efficacy.

The addendum to the final study report (Report ID 27963) deals with the development of the ratio of Gram-negative to Gram-positive organisms over the study period. On day -1, the proportion of Gram-negative bacteria in the subgingival flora was 71.1% in the Stomorgyl and 69.1% in the pradofloxacin group, which indicates the presence of periodontal disease in the sampled dogs. On day 7 and 28, Stomorgyl treatment had reduced the Gram-negatives to 47.5% and 52.8%, respectively. In the pradofloxacin group, post-treatment Gram-negative counts were 23.6% on day 7 and 25% on day 28. Hence, both treatments resulted in a significant reduction of subgingival Gram-negative bacteria over the study period. However, the reduction of Gram-negatives was significantly higher in the pradofloxacin than in the Stomorgyl group at all sampling time points (day -1 to day 7, day -1 to day 14 and day -1 to day 28; $p<0.001$). Bearing in mind that healthy periodontal flora consists of approximately 80% Gram-positive and 20% Gram-negative bacteria (up to 40% Gram-negatives may be seen in healthy geriatric dogs), the conclusion is that treatment with pradofloxacin returned the periodontal flora to a healthy state and stabilised this effect for at least three weeks after treatment. If a shift from a flora of mainly Gram-positive to one of mainly Gram-negative bacteria is indicative of periodontal disease (as discussed in the microbiology section of this Expert Report), the reversion of this change is clearly indicative of a return to periodontal health. If we

then remember that LPS, which play an important role in the pathogenesis of periodontal disease (direct cytotoxic effects, release of cytokines, systemic effects), are released by Gram-negative bacteria, a reduction of the Gram-negatives will lead to a reduction of LPS and LPS induced effects. Thus, the change of the composition of the periodontal flora induced by pradofloxacin is considered a very beneficial process and is the most important finding of this study. To my knowledge, pradofloxacin is the first antimicrobial, for which this beneficial effect could be shown in veterinary medicine.

In conclusion, pradofloxacin treatment significantly reduced loss of attachment, the total subgingival flora count, the rate of periopathogens and the proportion of Gram-negatives in the periodontal flora. These positive effects provided sufficient assurance that a dose of 3 mg/kg could be used in subsequent field studies with pradofloxacin in this indication.

B. Clinical field study

The results of the clinical field study with pradofloxacin have been reported by

Liège P (2004): Evaluation of the efficacy and safety of BAY 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions. Bayer Animal Health Report ID 27428.

A total of 131 dogs were included in the field study, 125 of which were also presented for examination at the end of the study. The per-protocol population consisted of 113 dogs. Fifty-nine were treated with pradofloxacin at a dose of 3 mg/kg b.w. once daily and 54 with Antirobe (clindamycin) at a dose of 5.5 mg/kg b.w. twice daily. Treatment duration was seven consecutive days in both groups. All animals were examined under general anaesthesia on study days 0 and 13, i.e. before and seven days after the end of treatment. Parameters assessed were periodontal pocket depth, bleeding on probing, reduction of total anaerobic subgingival count and other general clinical signs associated with periodontal disease (chewing ability, drooling and mouth pain).

The dogs were enrolled and examined by eleven veterinarians specialised in veterinary dentistry. All of them are members of the European Veterinary Dental College or the French GEROS (Groupe d'Etude et de Recherche en Odonto-Stomatologie). This ensured that the experienced colleagues correctly diagnosed the dogs and correctly assessed all study parameters, thereby reducing between-site variability.

Once periodontal disease has developed, the damage is irreversible, but can be arrested and controlled with suitable treatment (Hennet 1995b, Nielsen *et al.* 2000). Adjunctive antimicrobial therapy is an important element of such suitable treatment. However, with irreversible damage, curative treatment as for simple bacterial mono-infections is not possible in periodontal disease. What can be achieved by any kind of periodontal treatment, is an improvement of periodontal indices, e.g. a certain regain of attachment or reduction of gingivitis, and a more comfortable life for the animals by alleviation of general clinical symptoms such as pain (Harvey 1998). Hence, the study objective to look for such improvements by treatment with pradofloxacin was justified and correctly reflected by the use of the term "alleviation of clinical symptoms" in the study title instead of the misleading word "treatment".

The field study with pradofloxacin has used the so-called dirty tooth model, i.e. the teeth were not mechanically cleaned before antimicrobial treatment. It was appropriate to choose this model for various reasons. The dirty tooth model reveals the direct effects exerted solely by the antimicrobial drug without interference by scaling procedures and as such is suitable for direct comparison of the efficacy of two drugs for registration purposes. Furthermore, the clean tooth model, i.e. mechanical periodontal treatment followed by antimicrobial treatment, is only possible under laboratory conditions as all dogs need to be of the same breed and treated exactly in the same way. In a field study, however, there will be great variation in dog breeds, scaling procedures, diets and dental home care, all impairing comparability of study results obtained in a clean tooth model. Various breeds with different predisposition of plaque and calculus accumulation are normally included in a field study. There will be differences in scaling techniques between various investigators. Different diets will be used, some favouring plaque development, while others will have a plaque retarding effect. Compliance with dental home care is difficult to control. The clean tooth model requires long-term studies, with several examinations, all of which have to be conducted under general anaesthesia. This will reduce the level of owner compliance and result in relatively high exclusion rates. Hence, the dirty tooth model is the most suitable way to assess the effects of antimicrobials in periodontal disease under field conditions. Moreover, there is no reason to assume that an antimicrobial drug showing favourable results obtained under the more difficult conditions of a dirty tooth model should not induce similarly favourable results as an adjunct to mechanical therapy.

Periodontal pocket depth and bleeding on probing were measured on two target teeth, i.e. the maxillary left carnassial tooth and the maxillary right canine tooth. As periodontitis is a disease of the individual tooth rather than a disease of the animal (Harvey 1998) and can affect all teeth, only a few teeth or only one root of a multi-rooted tooth in a dog (Colmery and Frost 1986), it was justified to select target teeth diagnosed with periodontal disease for this study. The upper carnassials are usually most severely affected by periodontal disease (Hennet 1995b). We know that maxillary canine teeth are also commonly affected by periodontitis. Hence, appropriate target teeth were selected.

Dogs were included in the study if they showed moderate periodontal disease defined as presence of an inflamed gingival margin, pocket depth between 4 and 6 mm (for at least one pocket around one of the two target teeth) together with absence of furcation involvement and tooth mobility. Moderate periodontal disease has been correctly defined in the study protocol based on published literature (Colmery and Frost 1986, Nieves *et al.* 1997, Nielsen *et al.* 2000). In severe periodontitis, it would have been difficult to obtain a set of homogenous pocket depth measurements across the population of study animals, as dogs can either show deep periodontal pockets or pronounced loss of attachment by gingival recession without formation of a deep pocket (Colmery and Frost 1986). Hence, I agree with the statements in the discussion of study report ID 27428, that moderate periodontal disease was an appropriate choice necessary to reduce between-subject and between-site variability and also required to increase the statistical power of the analysis of efficacy. Comparability between groups was further enhanced by inclusion of dogs with true periodontal pockets of defined depths only. Furthermore, treatment of severe periodontitis often requires immediate surgical procedures or tooth extraction, which would have interfered with assessment of the study parameters. It was correctly pointed out in the discussion of the study report, that beneficial effects on clinical and microbiological periodontal parameters observed in moderate periodontitis will also be beneficial in severe periodontitis.

The study protocol initially contained a microbiological post-inclusion removal criterion, i.e. dogs had to be withdrawn from the study if the absence of one of the putative periopathogens *Porphyromonas gingivalis*, *Porphyromonas canoris*, *Porphyromonas cansulci*, *Porphyromonas cangingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Eikenella corrodens* and *Capnocytophaga ochracea* was confirmed. However, when the microbiological results became available, it was obvious that the goal to isolate one of these bacteria in most of the dogs had been too ambitious. Only 55% of the dogs were positive for periopathogens at that time. Consequently, the criterion was dropped by an amendment to the study protocol. This had no impact on the study results as periopathogens were not an efficacy criterion of the study. Nevertheless, attempts to isolate one of those bacteria were continued for each dog enrolled in the study. The problems encountered with bacteriological sampling of periodontal pockets have been discussed in this Expert Report. Under field conditions, increased transport times and variations of sampling procedures between different investigators might have further decreased the isolation rate of periopathogens.

The main efficacy criterion was mean pocket depth at the end of the study, and part of the secondary efficacy criteria were also related to periodontal pocket depth (maximum pocket depth, reduction of pocket depth). Periodontal pocket depth is a common parameter in studies on periodontal disease (Nielsen *et al.* 2000, Warrick *et al.* 2000). Reduction of periodontal pocket depth by treatment with an antimicrobial compound is the sum of regain of attachment and reduction of inflammatory gingival swelling. Both are very beneficial treatment results, as shallower pockets provide less space for accumulation of subgingival plaque and less favourable conditions for the establishment of aggressive anaerobic flora. The parameter Maximum Pocket depth was originally not included in the study protocol, but provided additional interesting information on the most severely diseased site around the target teeth. The assessment of pocket depth in this study was an appropriate choice as determination of loss of attachment under field conditions is problematic. Loss of attachment has to be determined from a fixed reference point, usually a small amalgam filling in the crown. However, most dog owners would refuse to have a hole drilled in the teeth of their dogs only for study purposes.

Periodontal pocket depth was measured with an electronic pressure sensitive probe. This is in line with the recommendation to use a constant force probe for measurements in the face of tissue inflammation because a rigid probe can penetrate 1 to 2 mm into the softened but still attached periodontal tissues (Harvey 1998). Again, I agree with the discussion of study report ID 27458 that the use of the pressure sensitive probe reduced both inter- and intra-investigator variability and thus increased the statistical power of the study. Furthermore, the electronic probe had an accuracy of ± 0.1 mm, resulting in far more accurate pocket depth values than the use of a manual probe with 1 mm increments. Pocket depth was measured on the buccal, mesial, distal and lingual side of the two target teeth. As measurements on the lingual side could have been influenced by the palatal ridges in small dogs, lingual probing depth was not included in the primary efficacy criterion.

The bleeding on probing score was recorded at the same time as pocket depth, buccal, mesial, distal and lingual on each target tooth. The probing sites were observed for 30 seconds after removal of the probe and bleeding was recorded according to a published scoring key (Warrick *et al.* 2000). Bleeding on probing is well correlated with the severity of gingivitis (Harvey 1998) and most likely a less subjective parameter than classical gingivitis scores.

Periodontal pockets of the maxillary right carnassial tooth and the maxillary left canine tooth were sampled with absorbent paper points for determination of the total subgingival anaerobic count. Two samples per tooth were taken and all four samples of a dog were pooled for analysis. Different target teeth were chosen for microbiology than for clinical assessment, because bleeding induced by probing would have diluted microbiological samples and reduced the viability of anaerobic bacteria, whereas microbiological sampling could have induced bleeding which would have interfered with assessment of bleeding on probing. Although total bacterial clearance cannot be expected from dental plaque and secluded periodontal pockets, a reduction of the total anaerobic count is a good indicator for activity against anaerobic bacteria, which are considered the most important periopathogens (Harvey 1998).

Both treatment groups were comparable before treatment with regard to breeds, age, gender, body weight, general condition, bleeding on probing, mean pocket depth and maximum pocket depth. Diets and access to chewing materials were very similar in both groups and were not considered to have influenced the study results.

For the main efficacy criterion mean pocket depth on day 13, a statistically significant difference was not detected between the treatment groups. However, using the Intent-to-treat (ITT) population, mean pocket depth tended to be significantly lower in the pradofloxacin group (*p=0.078415*). This difference was less pronounced when the per protocol (PP) population was used for analysis (*p=0.106218*). Non-inferiority of pradofloxacin to Antirobe was shown with *p=0.00139* and *p=0.00204* for the ITT and PP population, respectively.

The study report describes measures taken to reduce statistical background noise by exclusion of shallow pocket depths and outlier measurements. Shallow pocket depths were the result of probing a physiological sulcus on a tooth that showed true periodontal pockets at another probing site. As physiological gingival sulcus depth cannot be reduced by any periodontal treatment, it was justified to exclude pocket depth values of ≤ 2 mm. Outlier measurements were implausible differences between pocket depth values on day 0 and day 13, e.g. large reductions that were more likely to be due to probing errors rather than treatment effects. Even with a very accurate, pressure sensitive probe, it was not guaranteed that the investigators found exactly the same niche of the periodontal pocket upon probing on day 13. Hence, the exclusion of such implausible values was justified. The analysis of the corrected pocket depth data revealed that the close to significant initial difference in mean pocket depth in the ITT population was indeed induced by inclusion of shallow pocket depths and outlier measurements. This additional analysis could not detect any significant difference between the groups (*p=0.72802*). Hence an overestimation of product efficacy was avoided by using corrected pocket depth values. For this reason, the corrected pocket depth data were subsequently also used for the analyses of reduction of pocket depth and maximum pocket depth.

Both antimicrobials induced a statistically significant reduction of pocket depth over the study period. As for mean pocket depth, the reduction of pocket depth seemed to be significantly higher in the pradofloxacin group in the ITT population. However, this difference was no longer detected when corrected pocket depth values were employed in the analysis. Again, overestimation of treatment efficacy of pradofloxacin was adequately avoided by use of corrected pocket depth data. Pradofloxacin was non-inferior to Antirobe for the parameter reduction of pocket depth. The overall reduction of pocket depth induced by treatment with antimicrobial compounds in the field study may appear to be small at first glance. However, considering that the damage caused by periodontal disease is irreversible and progression can at best be stopped, any reduction of pocket depth is a positive effect demonstrating that treatment was able to aid in stopping the progression of disease. Pocket depth was reduced by 12% and 8% in the ITT population after pradofloxacin and clindamycin treatment, respectively. Using corrected pocket depth values, this reduction was 9% in the pradofloxacin and 8% in the Antirobe group. In the exploratory study (ID 27430), treatment with pradofloxacin reduced pocket depth by 13% compared to 9% for Stomorgyl (metronidazole + spiramycin). Using a clean tooth model, Nielsen *et al.* (2000) reported a 15% pocket depth reduction after adjunctive therapy with clindamycin compared to 3% induced by scaling alone. Although not detailed in the publication, this study most likely used the ITT population without pocket depth correction. Taking into account that both studies with pradofloxacin were conducted in the dirty tooth model, it can be concluded that the observed reduction of pocket depth was beneficial, almost comparable to reductions reported in a clean tooth model and all that can be achieved by one short treatment course with antimicrobials.

Maximum pocket depth was similarly reduced over the study period by both treatments. The reduction was 15% for pradofloxacin and 9% for Antirobe. Significant differences between the two treatment groups were not detected.

Pocket depth at the lingual probing site was reduced by 12% (maxillary left camassial) and 11% (maxillary right canine) in the pradofloxacin group. The corresponding reductions in the Antirobe group were 9% and 7%. Significant differences between the two treatment groups could not be shown for this parameter.

Bleeding on probing was significantly reduced by both treatments with *p=0.000003* for pradofloxacin and *p=0.000011* for Antirobe. Hence, both treatments were effective in reducing inflammation of gingiva and periodontal tissues. The bleeding on probing score was not significantly different between the treatment groups on day 13 of the study.

The general condition score composed of the parameters attitude, chewing ability, drooling and mouth pain was also significantly reduced by both antimicrobials demonstrating their beneficial effect on general clinical signs associated with periodontal disease. Translating these effects into daily practice means that treated dogs will generally be more comfortable and show fewer behavioural abnormalities. Again, both groups were statistically equivalent.

Treatment with pradofloxacin resulted in a significant reduction of the total anaerobic count between day 0 and day 13 of the study. A similar change could not be detected for Antirobe. The mean total anaerobic count was reduced by 80% in the pradofloxacin group compared to 8% in the Antirobe group. Hence, in contrast to Antirobe, pradofloxacin was effective in reducing the total anaerobic count, an important finding as anaerobic bacteria play a major role in development and progression of periodontal disease. A possible explanation for the observed difference is the bactericidal activity of pradofloxacin compared to the bacteriostatic mode of action of clindamycin.

The blinded investigators were asked to assess treatment efficacy for each dog at the end of the study. In the pradofloxacin group, efficacy was classified as very good in 14.1%, as good in 64.1% and as poor in 21.8% of the dogs. There was no significant difference to the Antirobe group, in which treatment effectiveness was classified as very good in 9.8%, as good in 65.6% and as poor in 24.6% of the animals. This shows that my colleagues and I were satisfied with the efficacy of antimicrobial treatment in 78% of the pradofloxacin treated dogs and 75% of the dogs treated with clindamycin.

In conclusion, both antimicrobial products exerted direct positive effects on the important periodontal parameters pocket depth and gingivitis (bleeding on probing), and on the associated clinical signs chewing ability, drooling and oral pain. Pradofloxacin induced a more pronounced reduction of the total anaerobic flora than clindamycin (Antirobe). Hence, both products were clinically equivalent with pradofloxacin having a microbiological advantage over clindamycin.

Conclusions

- Periodontal disease affects most dogs of more than five years of age. It is a disease of the individual tooth rather than a generalised disease of the complete dentition. Physiological and pathological pockets can be found at the same tooth at the same time.
- Periodontal disease is a severe inflammation of periodontal tissues caused by dental plaque resulting in progressive destruction of the periodontium and ultimate loss of the affected tooth.
- Periodontal disease is not caused by one or a few defined bacterial species, but is a complex polymicrobial infection that interacts with complex host defence mechanisms. It is characterised by a change from healthy periodontal flora mainly composed of Gram-positive non-motile cocci to the flora of periodontal disease dominated by aggressive anaerobic Gram-negative motile rods. *Porphyromonas* spp., *Prevotella* spp. and spirochetes are likely to be implicated in periodontal disease of dogs.
- Periodontal disease can have systemic consequences such as cardiovascular disease, endocarditis, pneumonia, stroke, as well as renal and hepatic disorders, all mediated via bacteraemia or LPS and cytokines being released into the bloodstream.

- There is no curative treatment of periodontal disease. However, progression of disease can be prevented by suitable periodontal treatment. Achievable objectives of periodontal therapy are reduction of inflammatory processes, moderate regain of attachment and stabilisation of a healthy periodontal flora.
- Mechanical periodontal therapy is, and will continue to be, the first-line treatment of periodontal disease.
- Under certain, well-defined conditions, broad-spectrum antimicrobials are an important part of periodontal therapy.
- Antimicrobials in periodontal disease should be used as an adjunct to mechanical cleaning.
- Pradofloxacin has a complete, broad-spectrum of activity against all relevant putative periodontal pathogens, which, in contrast to other systemic products registered for the indication periodontal disease, also includes Gram-negative aerobic bacteria.
- The exploratory study and clinical field study with pradofloxacin have utilised appropriate designs and assessed relevant periodontal parameters.
- Pradofloxacin exerted beneficial effects on the important clinical periodontal parameters pocket depth, loss of attachment and bleeding on probing. General clinical signs were alleviated. Pradofloxacin was able to re-establish and stabilise healthy periodontal flora over prolonged periods of time and to reduce the total subgingival anaerobic count.
- Pradofloxacin was clinically equivalent, but microbiologically superior to Stomorgyl (metronidazole + spiramycin) and Antirobe (clindamycin hydrochloride), both established and leading products in the treatment of periodontal disease.
- To my opinion, the favourable clinical and microbiological properties of pradofloxacin make it a promising alternative for the adjunctive antimicrobial therapy of periodontal disease in dogs.

References

Bell AF (1967): Dental disease in the dog. J. Small Anim. Pract. **6**, 421-428.

Black AP, Crichlow AM and Saunders JR (1980): Bacteraemia during ultrasonic teeth cleaning and extraction in the dog. Journal of the American Animal Hospital Association **16**, 611-616.

Colmery B, Frost P (1986): Periodontal disease. Etiology and pathogenesis. Vet. Clin. North Amer. Sm. Anim. Pract.**16**, 817-833.

Dajani AS, Bisno AL, Chung KJ, Durack DT, Freed M, Gerber MA, Karchmer AW, Millard HD, Rahimtoola S, Shulman ST, Watanakunakorn C, Taubert KA (1990): Prevention of bacterial endocarditis. Recommendations by the American Heart Association. J. Am. Med. Assoc. **12**, 2919-2922.

Dajani AS, Taubert KA, Wilson W, Bolger AF, Bayer A, Ferreri P, Gewitz MH, Shulman ST, Nouri S, Newburger JW, Hutto C, Pallasch TJ, Gage TW, Levison ME, Peter G, Zuccaro G (1997): Updated Guidelines. Prevention of bacterial endocarditis. Recommendations by the American Heart Association. Clin. Infect. Dis. **25**, 1448-1458.

DeBowes LJ (1998): The effects of dental disease on systemic disease. Vet. Clin. North Amer. Sm. Anim. Pract. **28**, 1057-1062.

DeBowes LJ, Mosier D, Logan E, Harvey CE, Lowry S, Richardson DC (1996): Association of periodontal disease and histologic lesions in multiple organs from 45 dogs. J. Vet. Dent. **13**, 57-60.

Eisenberg L, Suchow R, Coles RS, Deasy MJ (1991): The effects of metronidazole administration on clinical and microbiologic parameters of periodontal disease. Clin. Prevent. Dent. **12**, 28-34.

Fournier D, Mouton C, Lapierre P, Kato T, Okuda K, Ménard C (2001): *Porphyromonas gulae* sp. nov., an anaerobic, Gram-negative coccobacillus from the gingival sulcus of various animal hosts. International Journal of Systematic and Evolutionary Microbiology **51**, 1179-1189.

Gad T (1968): Periodontal disease in dogs. J. Periodont. Res. **3**, 268-272.

Golden AL, Stoller N, Harvey CE (1982): Survey of oral and dental diseases in dogs anaesthetized at a veterinary hospital. J. Am. Anim. Hosp. Assoc. **18**, 891-899.

Gordon J, Walker C, Hovliaras C, Socransky S (1990): Efficacy of clindamycin hydrochloride in refractory periodontitis: 24-month results. J. Periodontol. **61**, 686-691.

Gorrel C, Robinson J (1995): Periodontal therapy and extraction technique. *In* Crossley DA, Penman S (eds.): BSAVA Manual of Small Animal Dentistry, British Small Animal Veterinary Association, Cheltenham, UK, pp. 139-149.

Hamlin RL (1990): Identifying the cardiovascular and pulmonary diseases that affect old dogs. Vet. Med. **85**, 483-497.

Hamp SE, Olsson K, Farsø-Madsen K, Viklands P, Fornell J (1984): A microscopic and radiologic investigation of dental diseases of the dog. Vet. Radiol. **25**, 86-92.

Harvey CE (1998): Periodontal disease in dogs. Etiopathogenesis, prevalence, and significance. Vet. Clin. North Amer. Sm. Anim. Pract. **28**, 1111-1128.

Harvey CE, Shofer FS, Laster L (1994): Association of age and body weight with periodontal disease in North American dogs. J. Vet. Dent. **11**, 94-105.

Harvey CE, Shofer FS, Laster L (1996): Correlation of diet, other chewing activities and periodontal disease in North American client owned dogs. J. Vet. Dent. **13**, 101-105.

Harvey CE, Thornsberry C, Miller BR (1995): Subgingival bacteria – comparison of culture results in dogs and cats with gingivitis. J. Vet. Dent. **12**, 147-150.

Heijl L and Lindhe J (1980): Effect of selective antimicrobial therapy on plaque and gingivitis in the dog. J. Clin. Periodontol. **7**, 463-478.

Heimdahl A, Hall G, Hedberg M, Sanberg H, Söder PÖ, Tunér K, Nord CE (1990): Detection and quantitation by lysis-filtration of bacteremia after different oral surgical procedures. J. Clin. Microbiol. **28**, 2205-2209.

Hennet P (1995a): Dental anatomy and physiology of small carnivores. *In* Crossley DA, Penman S (eds.): BSAVA Manual of Small Animal Dentistry, British Small Animal Veterinary Association, Cheltenham, UK, pp. 93-104.

Hennet P (1995b): Periodontal disease and oral microbiology. *In* Crossley DA, Penman S (eds.): BSAVA Manual of Small Animal Dentistry, British Small Animal Veterinary Association, Cheltenham, UK, pp. 105-113.

Hennet P, Harvey CE (1991): Spirochetes in periodontal disease in the dog: a review. J. Vet. Dent. **8**, 16-17.

Isogai E, Isogai H, Miura H, Takano K, Aoi Y, Hayashi M, Namioka S (1989): Oral flora of mongrel and beagle dogs with periodontal disease. Jpn. J. Vet. Sci. **51**, 110-118.

Levin, J. (1999): Review of selected systemic, topical and local antibiotics for adjunctive nonsurgical periodontal therapy in dogs. Proc. Cent. Vet. Conf. 359-361.

Lindhe J, Hamp SE, Löe H (1975): Plaque induced periodontal disease in beagle dogs. A 4-year clinical, roentgenographical and histometrical study. J. Periodontol. Res. **10**, 243-255.

Loesche WJ (1999): Anaerobic periodontal infections as risk factors for medical diseases. Current Infectious Disease Reports **1**, 33-38.

Loesche WJ, Giordano JR, Hujoel P, Schwarcz J, Smith BA (1992): Metronidazole in periodontitis: reduced need for surgery. J. Clin. Periodontol. **19**, 103-112.

Marcella K (1988): Prophylactic antibiotic use in high-risk dentistry. Vet. Med. **83**, 646-647.

Miller BR, Harvey CE (1994): Compliance with oral hygiene recommendations following periodontal treatment in client-owned dogs. J. Vet. Dent. **11**, 18-19.

Nielsen D, Walser C, Kodan GK, Chaney RD, Yonkers T, VerSteeg JD, Elfring G and Slots J (2000): Effects of treatment with clindamycin hydrochloride on progression of canine periodontal disease after ultrasonic scaling. Vet. Therap. **1**, 150-158.

Nieves MA, Hartwig P, Kinyon JM and Riedesel DH (1997): Bacterial isolates from plaque and from blood during and after routine dental procedures in dogs. Vet. Surg. **26**, 26-32.

Pollmeier S (1994): [Efficacy of polishing after calculus removal and its influence on new formation of plaque and calculus in dogs] Der Einfluß von Politurmaßnahmen nach Zahnsteinentfernung auf die Neubildung von Plaque und Zahnstein im Hundegebiß. Veterinary dissertation, FU Berlin, Germany.

Riviere GR, Thompson AJ, Brannan RD, McCoy DE, Simonson LG (1996): Detection of pathogen-related oral spirochetes, *Treponema denticola*, and *Treponema socranskii* in dental plaque from dogs. J. Vet. Dent. **13**, 135-138.

Röcken FE, Pollmeier S, Fahrenkrug P, Trautvetter E (1996): [Efficacy of polishing after calculus removal and its influence on new formation of plaque and calculus in dogs] Der Einfluß von Polituramaßnahmen nach Zahnsteinentfernung auf die Neubildung von Plaque und Zahnstein im Hundegebiß. Prakt. Tierarzt **77**, 701-711.

Sarkiala E (1993): Treatment of periodontitis in dogs with tinidazole. J. Small Anim. Pract. **34**, 90-94.

Sarkiala E, Harvey CE (1993): Systemic antimicrobials in the treatment of periodontitis in dogs. Sem. Vet. Med. Surg. (Small Animals) **8**, 197-203.

Soames JV, Davies RM (1974): The structure of subgingival plaque in a beagle dog. J. Periodontal Res. **9**, 333-341.

Söder PÖ, Frithiof L, Wikner S, Wouters F, Engström PE, Rubin B, Nedlich U, Söder B (1990): The effect of systemic metronidazole after non-surgical treatment in moderate and advanced periodontitis in young adults. J. Periodontol. **61**, 281-288.

Tou SP, Adin DB, Castleman WL (2005): Mitral valve endocarditis after dental prophylaxis in a dog. J. Vet. Intern. Med. **19**, 268-270.

Walker C, Gordon J (1990): The effect of clindamycin on the microbiota associated with refractory periodontitis. J. Periodontol. **61**, 692-698.

Warrick JM, Inskeep GA, Yonkers TD, Stookey GK, Ewing TH (2000): Effect of clindamycin hydrochloride on oral malodor, plaque, calculus and gingivitis in dogs with periodontitis. Vet. Therap. **1**, 5-16.

Zetner K, Stoian C (2005): [Faster regeneration of the jaw bone following the application of a doxycycline containing polymer (Doxirobe) into intra-bony pockets]. Nachweis einer beschleunigten Regeneration des Kieferknochens durch die Applikation eines Doxycyclin-haltigen Polymers (Doxirobe) in Knochentaschen. To be published in Prakt. Tierarzt **86**, August 2005.

Zetner K and Thiemann G (1993): The antimicrobial effectiveness of clindamycin in diseases of the oral cavity. J. Vet. Dent. **10**, 6-9.

Curriculum vitae

Dr. Dr. Peter Fahrenkrug
Pinneberger Str. 43
25451 Quickborn
Germany

1952:	Born at Buxtehude, Lower Saxony, Germany
As of 1954:	Living in Quickborn, near Hamburg, Germany
December 1977:	Graduation from Hanover Veterinary School
June 1978:	Doctorate from Hanover Veterinary School (Dr. med. Vet.)
1978-1982:	Studies of human dentistry at Universities of Marburg and Hamburg
As of 1978:	Intensive studies and training in veterinary dentistry
1982:	Graduation from University of Hamburg and Doctorate in human dentistry (Dr. med. dent.)
1982-1985:	Positions as assistant dentist
1985-1994:	Dentist in own practice at Hasloh, district Pinneberg, Germany
1994:	Sale of dental practice
As of 1994:	Self-employed as veterinary dentist
	Consultant in veterinary dental therapy to various veterinary practices and clinics in Northern Germany
	Profound experience in veterinary dentistry in dogs and cats, pet animals, horses and exotic and zoo animals
	Certified specialist in veterinary dentistry including authorisation for continuing education
	Certified specialist in veterinary dentistry of horses
	Diplomate, EVDC (European Veterinary Dental College), 1998
	Conduct of continuing education of veterinary dentists
	Development and import of dental devices and instruments

	Adviser of HEILAND (Henry Schein Inc.), the leading international supplier of veterinary equipment; development of independent range and catalogue of veterinary dental equipment
1990-1996	Lecturer of veterinary dentistry, University of Berlin (Freie Universität)
As of 1983:	Regular lectures on veterinary dentistry in Germany (BPT-Seminars) and abroad; regular presentations and lectures at domestic and international conferences
	More than 570 lectures and seminars in veterinary dentistry and practice management, held in 27 countries in German, English, French and Spanish language
	More than 50 publications in German and international scientific journals and books
	Eleven educational videos on veterinary dentistry, one covering periodontal therapy
As of 1990:	Education and support of veterinarians in practice organisation and practice management

Memberships in veterinary scientific associations

Member of the BPT (Bundesverband Praktischer Tierärzte) – German Association of Practicing Veterinarians

Member of the DGK (Deutsche Gesellschaft für Kleintiermedizin) / GSAVA (German Small Animal Veterinary Association)

Member of the DVG (Deutsche Veterinärmedizinische Gesellschaft) – German Veterinary Medical Society

Member of the EVDS (European Veterinary Dental Society), charter vice president from 1991-1993, president from 1993 to 1995

Member of the EVDC (European Veterinary Dental College), co-chair of organising committee 1996-1998, diplomate since 1998

Member of the AVDS (American Veterinary Dental Society)

Fellow of the AVD (Academy of Veterinary Dentistry, USA)

Member of GEROS (Groupe d'Etude et de Recherche en Odonto-Stomatologie), CNVSPA, France

Member of the BVDA (British Veterinary Dental Association)

Member of Vet. Dent. Science e.V., Vienna, Austria

Charter-President, German Veterinary Dental Society, 2004

Veterinary political positions

State Veterinary Chamber Schleswig Holstein:

- Member of the committee for education and continuing education (1998-2004)
- Member of the committee for veterinary specialisation (2004-2008)
- Member of the committee for revision of the rules governing continuing education
- Member of the committee of examiners for exams to obtain the title "specialist in veterinary dentistry"
- Member of the committee of examiners for exams to obtain the title "specialist in dentistry in horses"

Member and vice chairman of the committee on veterinary medical fees of the German Veterinary Chamber (2000-2004).

Adviser of the German Veterinary Chamber in relation to veterinary medical fees for dental surgical procedures

Adviser of the German Veterinary Chamber and the State Veterinary Chambers in relation to the contents of the regulations governing specialisation in veterinary dentistry

Member of the committee of examiners for veterinary dental specialists of the State Veterinary Chambers of Lower Saxony, Baden-Wuertemberg, Berlin, North Rhine and Hamburg.

Member of the committee for continuing education of the State Veterinary Chambers of Lower Saxony, Baden-Wuertemberg, Berlin, North Rhine and Hamburg.

Member of the board of the BPT (Bundesverband Praktischer Tierärzte) – German Association of Practicing Veterinarians

Chairman of the Hamburg division of the BPT

Member of the board and international representative of the DGK (Deutsche Gesellschaft für Kleintiermedizin) – GSAVA (German Small Animal Veterinary Association)

President of the German Society for Veterinary Dentistry (Deutsche Gesellschaft für Tierzahnheilkunde) – 2004-2006

Secretary of the German-Croatian Veterinary Society

Member of the Advisory Committee of the Southern European Veterinary Conference, Barcelona, Spain

Editorial Assignments

Journal of Veterinary Dentistry – Member, Board of Reviewers, 1989-2000

Der Praktische Tierarzt (Germany) – Member, Scientific Advisory Board

Tierärztliche Praxis (Germany) – Reviewer publications on veterinary dentistry

Tierärztliche Umschau (Germany) - Reviewer publications on veterinary dentistry

Wiener Tierärztliche Monatsschrift (Austria) – Reviewer publications on veterinary dentistry

Selected publications

Fahrenkrug, P. 1978
Die Tierseuchensituation des schleswig-holsteinischen Landkreises Pinneberg in den Jahren 1954 bis 1977
[Epidemics of animals in the Pinneberg district, Schleswig Holstein, in the years 1954 to 1977]
Dissertation Veterinary Medicine, Hanover Veterinary School, 1978

Fahrenkrug, P. 1982
Stand und Möglichkeiten der Zahnheilkunde bei Haustieren
[State and possibilities of dentistry in domestic animals]
Dissertation, Dentistry, University Hospital Eppendorf, Hamburg, 1982

Fahrenkrug, P. 1984
Handbuch der Zahnbehandlung in der Kleintierpraxis , 1. Aufl.
[Handbook of dental treatment in small animal practice, 1st edition]
Distribution: A. Albrecht, Aulendorf, Germany

Fahrenkrug, P. 1985
Handbuch der Zahnbehandlung in der Kleintierpraxis , 2. Aufl.
[Handbook of dental treatment in small animal practice, 2nd edition]
Distribution: A. Albrecht, Aulendorf, Germany
ISBN 3-980049-0-7

Fahrenkrug, P. 1986
Handbuch der Zahnbehandlung in der Kleintierpraxis , 3. Aufl.
[Handbook of dental treatment in small animal practice, 3rd edition]
Distribution: A. Albrecht, Aulendorf, Germany
ISBN 3-980104-9-1-5

Fahrenkrug, P. 1987
Small animal Dentistry:
Introduction to equipment, methodology and endodontic principles
Tijdschrift voor Diergeneeskunde , **112** , Suppl.1, 25 S-33 S

Schmidt, H., u. P. Fahrenkrug 1987
Altersbedingte Pulpenveränderungen im Hundegebiß - anatomische Untersuchungen und klinische Konsequenzen
[Age related alteration of the pulpa in the canine dentition – anatomical investigations and clinical consequences]
Der Praktische Tierarzt **68**, 12-16

Heilmann, M. u. P. Fahrenkrug 1987
Zur Kieferorthopädie im Hundegebiß
[Orthodontics in the canine dentition]
Der Praktische Tierarzt **68**, 24-29

Fahrenkrug, P. 1987
Kieferorthopädische Behandlungsmöglichkeiten im Hundegebiß
[Orthodontic treatment options of the canine dentition]
Der Praktische Tierarzt **68**, 30-42

Fahrenkrug, P. 1987
Neue Instrumente für die Zahnbehandlung in der Kleintierpraxis
[New instruments for dental treatment in small animal practice]
Der Praktische Tierarzt **68**, 48-50

Fahrenkrug, P. 1987
Die Abdrucknahme im Hunde-/Katzengebiß
[How to take impressions of the canine and feline dentition]
Der Praktische Tierarzt **68**, 78-80

Weber, W. u. P. Fahrenkrug, 1987
Oro-nasale Fisteln nach Caninusverlust im Oberkiefer des Hundes
[Oro-nasal fistulas subsequent to the loss of canine teeth in the upper jaw of dogs]
Der Praktische Tierarzt **68**, 83-84

Fahrenkrug, P. 1988
Handbuch der Zahnbehandlung in der Kleintierpraxis , 4 Aufl.
[Handbook of dental treatment in small animal practice, 4th edition]
Distribution: A. Albrecht, Aulendorf, Germany
ISBN 3-980104-9-2-3

Fahrenkrug, P. 1988
Tierzahnheilkunde - quo vadis?
[Veterinary dentistry – quo vadis?]
Der Praktische Tierarzt **69**, 5-8

Fahrenkrug, P. 1988
Zur Wirtschaftlichkeit der Tierzahnheilkunde
[Economical aspects of veterinary dentistry]
Der Praktische Tierarzt **69**, 57-62

Fahrenkrug, P. 1991
Les Protheses dentaires
Recueil De Medecine Veterinaire, **167**, 1079 – 1089

Shipp, A. D., u. P. Fahrenkrug, 1992
Practitioner's Guide to Veterinary Dentistry
Dr. Shipp's Laboratories, Beverly Hills, USA
ISBN 0-9635578-0-7

Fahrenkrug, P. 1993
Krankheiten der Zähne
[Diseases of the teeth]
in: Wiesner, E. (Hrsg.) - Kompendium der Heimtierkrankheiten 2
[in: Wiesner, E. (edt.) – compendium of pet animal diseases 2]
Gustav Fischer Verlag, Stuttgart, Jena, New York

Fahrenkrug, P., 1994
Milchzahn- und Zahnwechselprobleme des Junghundes
[Problems of deciduous teeth and change of teeth in young dogs]
Veterinärspiegel **4/94**, 12-24

Röcken, F. E., S. Pollmeier, P. Fahrenkrug, E. Trautvetter, 1996
Der Einfluß von Politurmaßnahmen nach Zahnsteinentfernung auf die Neubildung von Plaque und Zahnstein im Hundegebiß
[Efficacy of polishing after calculus removal and its influence on new formation of plaque and calculus in dogs]
Der Praktische Tierarzt, **77**, 701-711

Röcken F. E., P. Fahrenkrug, 1996
Zur Behandlung des Caninussteilstandes im Unterkiefer beim Hund
[Treatment of base narrow canine teeth in the lower jaw of the dog]
Der Praktische Tierarzt, **77**, 733-743

Fahrenkrug, P. 1996
Krankheiten der Zähne und des Kiefers
[Diseases of the teeth and jaws]
in: Kraft,W. u. U.M. Dürr (Hrsg/edts.)
Katzenkrankheiten, 4. Auflage. [Diseases of the cat, 4th edition].
M .u. H. Schaper, Hanover, 439-466

Fahrenkrug, P., F. San Roman, U. Thams, J. I. Trobo, F. Munoz, M. A. Vives 1998
Prostodoncia
in: San Roman, F. (edt.)
Atlas de Odontologia en Pequenos Animales
Grass Ediciones/Editores Medicos, Madrid, Spain, 185-199

Fahrenkrug, P. 1998
In: Verstraete, Frank J. M.
Self-Assessment Colour Review of Veterinary Dentistry
USA: Iowa State University Press
GB: Manson Publishing

Fahrenkrug, P. 2003
Krankheiten der Zähne und des Kiefers
[Diseases of the teeth and jaws]
in: Kraft, W., U. M. Dürr u. K. Hartmann (Hrsg./edts.)
Katzenkrankheiten – Klinik und Therapie 5. Auflage, [Diseases of the cats – clinical features and therapy]
M. u. H. Schaper, Hanover, 560-591

Fahrenkrug, P. 2003
Die systematische Parodontalbehandlung in der Tierarztpraxis – medizinische und wirtschaftliche Aspekte
[Systematic periodontal therapy in veterinary practice – medical and economical aspects]
Bpt-info, Mitgliederzeitschrift des Bundesverbandes praktizierender Tierärzte
[Bpt-info, journal for the members of the German Association of Practising Veterinarians]
Part 1: July 2003, 8-11
Part 2: September 2003, 11-13
Part 3: October 2003, 9-10

Fahrenkrug, P. 2003
Stomatologischer Untersuchungsgang bei Hund und Katze
[Stomatological examination of dogs and cats]
Kapitel 2.8 in Kramer, M.: Kompendium der allgemeinen Veterinärchirurgie
[Chapter 2.8 in Kramer, M.: Compendium of general veterinary surgery]
Schlütersche Verlagsanstalt Hannover, 64-76

Fahrenkrug, P. 2003, 2004
Die systematische Parodontalbehandlung in der Tierarztpraxis – besonders wichtig für ältere Patienten!
[Systematic periodontal therapy in veterinary practice – particularly important for older patients]
Praxis nah, Monatszeitschrift für Tierarzthelferinnen des Berufsverbandes der Arzt-, Zahnarzt- und Tierarzthelferinnen BdA – drei Teile
[Praxis nah, monthly journal of the professional association of medical, dental and veterinary nurses] – three parts

In preparation

The History of equine Dentistry
In: Baker, G.B., J. Easley (edts.). Equine Dentistry, 3rd edition.
W. B. Saunders, London, Edinburgh, New York, Philadelphia, Sydney, Toronto

Chapter on dental diseases
In: Grünbaum and Schimke : Klinik der Hundekrankheiten 3.Aufl. [Clinical features of canine diseases]
Approximately 4th quarter 2005

Fahrenkrug, P.
Farbatlas der Zahnbehandlung in der Kleintierpraxis
[Colour atlas of dental treatment in small animal practice]
Schlütersche Verlagsanstalt, Hanover, ca. 2006

Fahrenkrug, P., P. Stelzer
Pferdezahnheilkunde [Dentistry in the horse]
Parey Verlag / Blackwell

Lectures, seminars, presentations at conferences

Guest lectures at the following universities

Veterinary College of Chile – Santiago de Chile
Universidad Austral, Faculty of Veterinary Sciences – Valdivia, Chile
Philipps University, Marburg, Germany
Medical Academy, Erfurt, Germany
Small Animal Clinic, Hanover Veterinary School, Germany
École Nationale Veterinaire de Nantes, France
University Clinic Würzburg, Germany
Christian Albrechts University, Kiel, Germany
Veterinary Faculty of the University of Bern, Switzerland
École Nationale Veterinaire, Alfort, France
University of Berlin (Freie Universität), Germany
University of Luxembourg, Luxembourg
University of Auburn, Veterinary Faculty, Auburn, Alabama, USA
University of California, Davis, USA
University of Giessen, Veterinary Faculty, Germany
Veterinary and Pharmaceutical University, Brno, Czech Republic

Seminars

More than 260 seminars for the BPT in Germany, mainly two day weekend training courses covering all aspects of veterinary dentistry from basic level to master classes

More than 20 seminars on veterinary periodontal therapy

Regular seminars for continuing education of veterinary nurses

Seminars abroad held in Austria, Czech Republic, Denmark, France, Greece, Italy (South Tyrol), Luxembourg, Portugal, Spain, Switzerland and USA

Presentations at conferences

More than 300 presentations as invited speaker at national and international conferences including key note speeches, plenary lectures and state of the art lectures

More than 35 presentations on periodontal disease and periodontal therapy

A selection of international conferences is listed below:

Veterinary Dentistry 87, Cincinnati, USA, 1987

Vetadontics 88, New Orleans USA, 1988

1st Annual Meeting of the British Veterinary Dental Association (BVDA), Harrogate, UK, 1989; presentations at further meetings in 1992, 1993, 1994, 1995, 1996, 2005

Vetadontics 89, New Orleans, USA, 1989

Annual Meeting of GEROS (Groupe d'Etude et de Recherche en Odontostomatologie), Paris, 1989; presentations at further meetings in 1990, 1993, 1996

XVI. World Congress of the World Small Animal Veterinary Association (WSAVA), Vienna, Austria, 1991

2nd World Veterinary Dentistry Congress, Vienna, Austria, 1991

XVII. World Congress WSAVA, Rome, Italy, 1992

Veterinary Dental Forum, Las Vegas, USA, 1992

XVIII. World Congress WSAVA, Berlin, Germany, 1993

XV. National Symposium, SAVAB, Brussels, Belgium, 1993

Veterinary Dentistry 94, Philadelphia, USA, 1994

XIX. World Congress WSAVA, Durban, South Africa, 1994

IADR – International Association for Dental Research, 32nd Meeting, Ljubljana, Slovenia, 1995

2nd European Congress of FECAVA, Brussels, Belgium, 1995

XXI. World Congress WSAVA, Jerusalem, Israel, 1996

10th Annual Veterinary Dental Forum, Houston, USA, 1996

World Veterinary Dental Congress, Birmingham, UK, 1997

11th Annual Veterinary Dental Forum, Denver, USA, 1997

The North American Veterinary Conference, Orlando, USA, 1998

10th Annual Meeting of the Hungarian Small Animal Veterinary Association, Budapest, Hungary, 2001

XXIX. World Congress WSAVA, Rhodes, Greece, 2004

The North American Veterinary Conference, Orlando, USA, 2005

TABULATED STUDY REPORT ID 27420

Company Name:	Bayer Animal Health GmbH	**Reference Number: 16**	Page 1 of 2
Product:	Veraflox Tablets Veraflox Oral Suspension	Study period: 2004	
Active Substance:	Pradofloxacin	Report issue date: 30.04.2004 Report number: ID 27420	

Title: Comparative activity of pradofloxacin and other fluoroquinolones against anaerobic bacteria isolated from dogs and cats: determination of Minimum Inhibitory Concentration (MIC)

Test Substance: Pradofloxacin, marbofloxacin, difloxacin and ibafloxacin

Test organisms:	141 anaerobic bacteria isolated in the UK during the period 2000-2002	
Methodology:	Standardised NCCLS methods	
Formulations: **Test substances:**	Pradofloxacin, marbofloxacin, difloxacin and ibafloxacin (concentrations adjusted to base and for purity)	
Stock vehicle:	Deionised water adjusted with sodium hydroxide, when necessary to aid dissolution	
Dilutions: **Final concentrations:**	Sterile deionised water, final dilution in culture medium 0.016, 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 µg/ml	
Media and Test Plates:	Fastidious anaerobe agar was used for inoculum preparation and supplemented Brucella Blood Agar for MIC testing	
Inoculum:	Overnight cultures adjusted to 0.5 McFarland Standard. 5 µl of standardised bacterial suspension containing 1×10^8 cfu/ml achieves an approximate inoculum level of 5×10^5 cfu per inoculum site.	
Replicates:	1	
Incubation:	35 ± 1° C for 24 ± 1hour, if strains did not grow sufficiently to allow reading of MIC results after 24 h, plates were re-incubated and inspected daily until growth was sufficient to permit interpretation.	
Calculations:	MIC	lowest concentration which completely inhibits growth
	MIC_{50}	minimum concentration which inhibits 50 % of strains within a genus
	MIC_{90}	minimum concentration which inhibits 90 % of strains within a genus
	Geometric mean	mean of all single observations

Study conducted by the applicant: No
If 'no' indicate the name and address of the institute that conducted the study: Don Whitley Scientific, 14 Otley Road, Shipley, West Yorkshire, BD17 7SE, UK

Study in compliance with GLP: Yes

Company Name: Bayer Animal Health GmbH	**Reference Number: 16**	Page 2 of 2
Product: Veraflox Tablets Veraflox Oral Suspension	Study period: 2004	
Active Substance: Pradofloxacin	Report issue date: 30.04.2004 Report number: ID 27420	

Title: Comparative activity of pradofloxacin and other fluoroquinolones against anaerobic bacteria isolated from dogs and cats: determination of Minimum Inhibitory Concentration (MIC)

Test Substance: Pradofloxacin, marbofloxacin, difloxacin and ibafloxacin

Results:

Bacterial Genus (No. of strains)	*Antimicrobial*	Summary MIC parameters (µg/ml)			
		MIC range	MIC_{50}	MIC_{90}	Geometric Mean
Bacteroides (29)	Pradofloxacin	0.062 - 1	0.25	1	0.3
	Marbofloxacin	0.062 - 8	1	4	1.1
	Difloxacin	0.25 - 16	2	8	2.0
	Ibafloxacin	0.25 - 32	8	32	4.6
Clostridium (32)	Pradofloxacin	0.062 - 2	0.25	0.5	0.3
	Marbofloxacin	0.25 - 8	1	2	0.9
	Difloxacin	0.25 - 16	2	8	1.5
	Ibafloxacin	0.125 - 8	4	4	1.9
Fusobacterium (22)	Pradofloxacin	0.031 - 2	0.5	1	0.5
	Marbofloxacin	0.25 - 64	4	64	4.7
	Difloxacin	0.25 - 16	2	16	3.8
	Ibafloxacin	0.5 - 64	4	32	5.7
Porphyromonas (6)	Pradofloxacin	0.062 – 0.5	0.062	Not applicable	0.1
	Marbofloxacin	0.5 - 8	0.5		0.8
	Difloxacin	0.5 - 1	1		0.9
	Ibafloxacin	0.25 - 16	16		8.0
Prevotella (20)	Pradofloxacin	≤ 0.016 - 1	0.25	1	0.2
	Marbofloxacin	0.25 - 8	1	4	1.0
	Difloxacin	0.125 - 32	1	16	1.5
	Ibafloxacin	1 - 32	8	16	7.5
Propionibacterium (5)	Pradofloxacin	0.125 - 1	0.25	Not applicable	0.3
	Marbofloxacin	0.5 - 4	1		1.2
	Difloxacin	1 - 16	2		2.6
	Ibafloxacin	2 - 8	4		4.0
Sporomusa (6)	Pradofloxacin	≤ 0.016 - 1	0.25	Not applicable	0.2
	Marbofloxacin	0.062 - 4	0.5		0.6
	Difloxacin	0.125 - 16	1		2.2
	Ibafloxacin	0.25 - 32	2		2.0
All strains (141)	Pradofloxacin	≤ 0.016 - 2	0.25	1	0.3
	Marbofloxacin	0.062 - 64	1	8	1.2
	Difloxacin	0.062 - 32	2	16	1.9
	Ibafloxacin	0.062 - 64	4	16	3.6

This table includes only those genera represented by at least 3 bacterial strains.
MIC_{50} is calculated only for genera containing at least 10 bacterial strains.

TABULATED STUDY REPORT ID 27430 / ID 27963

Company Name:	Bayer Animal Health GmbH	Page 1 of 2
Product:	Veraflox Tablets	Study period:
Active Substance:	Pradofloxacin	Report issue date: 28.04.2003 Report number: ID 27430 **Ref. 68** Report issue date: 24.11.2003 Report number: ID 27963 **Ref. 69**

Title: Exploratory study on the activity of BAY 14-1877 tablets after oral administration on sub-gingival flora in cases of canine periodontal disease (ID 27430)

Addendum to the final study report 142.089 and 142.090 entitled: Exploratory study on the activity of BAY 14-1877 tablets after oral administration on sub-gingival flora in cases of canine periodontal disease (ID 27963)

Test Substance: Pradofloxacin

Methodology:

In a controlled, blinded, non-GCP study conducted to confirm the efficacy of Veraflox Tablets in the treatment of periodontal infections induced by sub-gingival flora, female dogs with clinical signs of periodontal disease were treated with either pradofloxacin at a dose rate of 3 mg/kg body weight once daily for six consecutive days, or Stomorgyl twice daily at a dose of 12.5 mg/kg body weight of metronidazole plus 75,000 I.U./kg of spiramycin for six days. Eight dogs were included per group and four periodontal sites were selected for assessment in each dog.

Results:

The degree of loss of attachment and the total bacterial population decreased significantly in both groups over the study period. Although there were no significant differences between the treatment groups, this reduction was greater in the pradofloxacin group with 0.47 mm compared to 0.32 mm in the Stomorgyl group.

Both treatments significantly reduced the total anaerobic count and the proportion of periodontopathogens over the study period, but Stomorgyl had a significant advantage over pradofloxacin in terms of the number of periodontopathogens isolated after treatment. However, repeated isolation of specific periopathogenic bacteria is very difficult and unreliable. Even sampling of the same pocket at different times cannot be guaranteed. Hence, this finding might have well been due to sampling errors and is far less important than the beneficial effects on periodontal flora and loss of attachment observed after pradofloxacin treatment.

The most important observation was made by means of additional analysis of the ratio of Gram-negative to Gram-positive bacteria in the periodontal pockets. The major periodontal pathogens are black-pigmented Gram-negative anaerobic rods. The statistical analysis of treatment effect on Gram-positive and Gram-negative bacteria revealed that a reduction in the proportion of Gram-negative bacteria was seen in both groups over the study period. However, the reduction was significantly greater in the pradofloxacin treated group over the 28-day study period, thus, indicating that pradofloxacin treatment had a lasting stabilising effect on the beneficial periodontal bacterial flora.

Company Name:	Bayer Animal Health GmbH	Page 2 of 2
Product:	Veraflox Tablets	Study period:
Active Substance:	Pradofloxacin	Report issue date: 28.04.2003 Report number: ID 27430 **Ref. 68** Report issue date: 24.11.2003 Report number: ID 27963 **Ref. 69**

Title: Exploratory study on the activity of BAY 14-1877 tablets after oral administration on sub-gingival flora in cases of canine periodontal disease (ID 27430)

Addendum to the final study report 142.089 and 142.090 entitled: Exploratory study on the activity of BAY 14-1877 tablets after oral administration on sub-gingival flora in cases of canine periodontal disease (ID 27963)

Test Substance: Pradofloxacin

Conclusions:

In conclusion, pradofloxacin at a dose of 3 mg/kg once daily was clinically equivalent and, in terms of its beneficial stabilising effect, microbiologically superior to a well-established control product. Thus, this study provides sufficient assurances to confirm that, at a dose of 3 mg/kg body weight, Veraflox Tablets when administered once daily can be expected to be efficacious in the adjunctive treatment of periodontal disease in the dog.

Study conducted by: Applicant

Study in compliance with GCP: No

TABULATED STUDY REPORT ID 27428

Company Name: Bayer Animal Health GmbH		**Ref. 76**	Page 1 of 10
Product name: Veraflox Tablets		**Active substance:** Pradofloxacin	
Title:	Evaluation of the efficacy and safety of Bay 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions		
Report ID:	27428	**Report Issue Date:**	19/04/2004
Study type:	Clinical field study	**GCP/GLP**	GCP

Study design			
Objectives:	To confirm the efficacy and safety of Bay 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs. Efficacy was compared to that of ANTIROBE capsules.		
Species:	Dogs	**Breed:**	Mixed
Age:	Group I = 7.56 ± 2.95 years Group II = 7.38 ± 2.77 years	**Bodyweight:**	Group I = 18.04 ± 11.98 kg Group II = 18.14 ± 11.48
Number/sex:	64 females; 61 males		
Study dates:	04/07/2002 – 15/07/2003		
Location:	13 veterinary practices in Europe: Belgium (1), France (7), Germany (2), Italy (2), Netherlands (1).		
Procedure:	Controlled, randomised, blinded, multicentre field study. 127 dogs were included in the study and randomly allocated to treatment group. At each site, half the dogs were treated with Bay 14-1877 and the remaining half with ANTIROBE. For each animal the study started on Day 0. Treatment was given from Day 0 to Day 6. Seven days after termination of treatment (Day 13 ± 1), all animals were re-examined in order to assess final response to treatment.		
Important inclusion/ exclusion criteria:	Inclusion criteria: - Dogs diagnosed with moderate periodontal disease; - Dogs showing inflammation of the gingival margin of the target teeth and periodontal pocket depth (≥ 4 mm) for at least 1 probing site. Exclusion criteria: - Dogs not showing inflammation of the gingival margin or with periodontal pocket depth < 4 mm at all probing sites; - Dogs diagnosed with severe periodontitis; - Dogs showing gingival hyperplasia; - Dogs showing calculus that would have prevented reproducible periodontal probing; - Dogs showing stomatitis unrelated to periodontal disease; - Dogs that underwent recent dental cleaning (less than 3 months).		

Dose form:

Treatment	Form	Route	Dose	Duration
Group I Bay 14-1877	Tablets containing 15, 60 or 120 mg pradofloxacin*	Oral	3 mg/kg b.w. sid[1]	7 days (D0 to D6)
Group II ANTIROBE	Capsules containing 25, 75 or 150 mg clindamycin hydrochloride*	Oral	5.5 mg/kg b.w. bid[2]	7 days (D0 to D6)

* Tablet size dependent on bodyweight
[1] Mean actual dose administered = 3.78 ± 0.70 mg/kg b.w.
[2] Mean actual dose administered = 8.86 ± 2.60 mg/kg b.w.

Title:	Evaluation of the efficacy and safety of Bay 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions		
Report ID:	27428	**Report Issue Date:**	19/04/2004
Study type:	Clinical field study	**GCP/GLP**	GCP

Parameters monitored:

Periodontal parameters

Diagnosis of the periodontal condition was based on the clinical examination of the 2 maxillary canine teeth and the 2 maxillary carnassials (4th premolars). The assessment of pocket depth (PD) and bleeding on probing (BOP) was performed on the upper left carnassial and the upper right canine tooth. Each of the 2 target teeth was probed on 4 sites for pocket depth: distal, buccal, mesial and lingual. The BOP was assessed immediately after the measurement of periodontal pocket depth. BOP was scored according to an objective scoring system. The score increased with severity of bleeding.

General Condition

This was evaluated according to an objective scoring system that considered the following criteria: attitude, chewing ability/appetite, drooling and mouth pain. The scores for each criterion were added to form a total General Condition Score (GCS), which could range between 0 and 12. The GCS increased with severity of condition.

Investigator's assessment of efficacy

This was assessed according to a 4-point scale (very good, good, poor, very poor).

Investigator's assessment of acceptability

This was assessed according to a 4-point scale (very good, good, poor, very poor).

Observation frequency:

Parameter	Day(s) of observation
Clinical examination	Day 0 and Day 13 ± 1
General condition (GCS)	Day 0 and Day 13 ± 1
Assessment of pocket depth (PD)	Day 0 and Day 13 ± 1
Assessment of bleeding on probing (BOP)	Day 0 and Day 13 ± 1
Bacteriological examination	Day 0 and Day 13 ± 1
Investigator's assessment of efficacy	Day 13 ± 1
Investigator's assessment of acceptability	Day 13 ± 1

Bacterio-logical examination:

Bacteriological examinations were performed on Day 0 and Day 13 ± 1. Two samples were taken from a periodontal pocket of the maxillary left canine and the maxillary right carnassial. The 4 samples were pooled for microbiological analysis. The main aims of the bacterial examinations were to:

- Quantify the total sub-gingival flora;
- Confirm the presence of 1 or more of the following putative periodontal pathogens: *Porphyromonas gingivalis*, *Porphyromonas canoris*, *Porphyromonas cansulci*, *Porphyromonas cangingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Eikenella corrodens* and *Capnocytophaga ochracea*;
- Assess the *in vitro* susceptibility (MIC) of the pathogens isolated on Day 0 to Bay 14-1877 and clindamycin (Report ID: 27429).

Product name: Veraflox Tablets		**Active substance:** Pradofloxacin	
Title:	Evaluation of the efficacy and safety of Bay 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions		
Report ID:	27428	**Report Issue Date:**	19/04/2004
Study type:	Clinical field study	**GCP/GLP**	GCP

Clinical efficacy parameters:	**Primary efficacy criterion:** - Mean periodontal pocket depth of both target teeth on Day 13 ± 1. **Secondary parameters for treatment efficacy:** - Reduction of pocket depth over the study period, taking into account the factors treatment, tooth and probing site; - Comparison of pocket depth at the lingual probing site of the maxillary left carnassial on Day 13 ± 1; - Comparison of pocket depth at the lingual probing site of the maxillary right canine on Day 13 ± 1; - Reduction of mean bleeding on probing (BOP) score (mean of both target teeth) over the study period; - Comparison of mean BOP score at the maxillary left carnassial on Day 13 ± 1; - Comparison of mean BOP score at the maxillary right canine on Day 13 ± 1; - Change in mean General Condition Score (GCS) over the study period; - Reduction of total sub-gingival flora count over the study period; - Investigators' assessment of efficacy on Day 13 ± 1.	
Safety:	Adverse Events (AE) and Serious Adverse Events (SAE) were recorded.	
Statistical analysis methods:	The experimental unit was the individual animal. Only statistical tests giving $p < 0.05$ were accepted as significant (type 1 error α=5%). The PP population was used in all statistical tests for treatment efficacy while the ITT population was used to confirm only the results of the statistical test on the main efficacy criterion. To remove background noise from the statistical tests, pairs of outlier measurements and shallow pocket depths were removed from the dataset.	
	Variable	**Statistical tests**
	Group comparability	
	Group comparability before treatment (Intent-To-Treat population)	Fisher's exact test, Student t-test, Mann-Whitney U-test, depending on variable
	Primary efficacy criterion	
	Comparison of mean pocket depth of both target teeth between Groups I and II	Student t-test (symmetric) and non-inferiority test using an upaired Student t-test (asymmetric). The non-inferiority limit (θ) was set as 5% of PD_{II} (mean pocket depth from the gingival margin recovery rate in Group II (ANTIROBE).
	Secondary efficacy criteria	
	Comparison of maximum pocket depth (PD) between groups	Student t-test
	Comparison of mean reduction in PD between groups ($PD_0 - PD_{13}$). Interactions between treatment, tooth and time factors were investigated. Reduction of pocket depth was compared between teeth, probing sites and groups	Student t-test, Mann-Whitney U-test, 3-way ANOVA on repeated measures, non-inferiority test (θ = PD reduction of 0.2 mm)
	Comparison of pocket depth at both lingual probing sites on Day 13 ± 1 between groups	Student t-test
	Comparison of BOP score on Day 13 ± 1 between groups. The BOP scores were pooled for both teeth	Mann-Whitney U-test. The reduction in BOP score was compared between groups using a Wilcoxon W test

Title:	Evaluation of the efficacy and safety of Bay 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions		
Report ID:	27428	**Report Issue Date:**	19/04/2004
Study type:	Clinical field study	**GCP/GLP**	GCP

Statistical analysis methods continued:	Comparison of GCS between groups	Mann-Whitney U-test
	Comparison of log_{10} bacterial counts between groups and times (Days 0 and 13 ± 1)	2-way ANOVA on repeated measures
	Comparison of investigators' assessment of efficacy between groups	Mann-Whitney U-test
	Comparison of investigators' assessment of acceptability between groups	Mann-Whitney U-test

Pre-treatment results: The dogs in both groups consisted of similar breeds and were comparable for age, sex ratio and bodyweight. In addition the two groups were comparable as far as efficacy variables are concerned.

Summary of animal characteristics and group comparability prior to treatment

Parameter	Group I Bay 14-1877	Group II ANTIROBE	p-value
AGE (years)			
Mean (SD)	7.56 (2.95)	7.38 (2.77)	Student t-test p=0.725760
Median	7.38	7.13	
Min - Max	1.25-17.56	2.10-13.45	
Parameter	**Group I Bay 14-1877**	**Group II ANTIROBE**	**p-value**
SEX			
Male	32 (50.0%)	29 (47.5%)	Fisher's exact test p=0.858526
Female	32 (50.0%)	32 (52.5%)	
STERILISATION			
No	47 (73.4%)	44 (72.1%)	Fisher's exact test p=1.0
Yes	17 (26.6%)	17 (27.9%)	
BODYWEIGHT (KG)			
Mean (SD)	18.04 (11.98)	18.14 (11.48)	Student t-test p=0.961037
Median	16.55	17.30	
Min - Max	2.55-50	2.5-46	
GENERAL CONDITION SCORE			
Mean (SD)	0.64 (1.36)	0.52 (1.25)	Mann-Whitney U-test p=0.882038
Min - Max	0-8	0-8	
BLEEDING ON PROBING SCORE			
Mean (SD)	0.76 (0.68)	0.70 (0.57)	Mann-Whitney U-test p=0.883764
Median	0.625	0.625	
Min - Max	0-3	0-3	
MEAN POCKET DEPTH IN MM (ITT POPULATION)			
Mean (SD)	3.47 (0.57)	3.56 (0.63)	Student t-test p=0.427014
Median	3.49	3.62	
Min - Max	2.07-4.78	2.22-5.33	
MAXIMUM POCKET DEPTH (mm)			
Mean (SD)	4.76 (1.07)	4.90 (0.92)	Student t-test p=0.434050
Median	4.4	4.6	
Min - Max	2.5-10.6	3.3-8.0	

Product name: Veraflox Tablets | **Active substance:** Pradofloxacin

Title: Evaluation of the efficacy and safety of Bay 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions

Report ID: 27428 | **Report Issue Date:** 19/04/2004

Study type: Clinical field study | **GCP/GLP** | GCP

Study results:

The Intent-to-Treat (ITT) population consisted of 125 dogs. 12 of the dogs in the ITT population were not included in the Per-Protocol (PP) population, which therefore consisted of 113 dogs.

PRIMARY EFFICACY CRITERION

Comparison of mean pocket depth (PD) in mm between Groups I and II on Day 13 ± 1: ITT population (n=125 dogs)

Mean PD (mm)	Group I Bay 14-1877 (n=64)	Group II ANTIROBE (n=61)	p-value
Mean (SD)	3.06 (0.74)	3.29 (0.69)	Student t-test p=0.078415
Median	3.16	3.38	
Min - Max	1.17-4.47	1.73-4.95	

Mean PD on Day 13 ± 1 tended to be higher in Group II (ANTIROBE) than in Group I (Bay 14-1877). The difference was slightly above the significance level (p=0.078415).

Non-inferiority test for mean pocket depth (mm) on Day 13 ± 1: ITT population

Descriptive statistics

PD (mm)	Mean	Standard deviation	Standard error	95% LCL of mean	95% UCL of mean
Group I n=64	3.059115	0.7381452	9.23E-02	2.874731	3.243498
Group II n=61	3.286885	0.6946096	8.89E-02	3.108988	3.464783

T-Test (equal-variance and normality assumptions were met)

Alternative hypothesis	t-value	Prob level	Decision (α=0.05)	Power (α=0.05)	Power (α=0.01)
Difference < 0.164 mm	-3.0526	0.00139*	Reject H_0	0.917869	0.755715

Difference: $PD_I - PD_{II}$; Significance level: α=0.05; Clinically Significant Difference: Θ=5% of PD_{II} or 0.164 mm; * Statistically significant difference (p<0.05)

For the ITT population, the inferiority assumption was rejected at the level of p=0.00139. Therefore, BAY 14-1877 was non-inferior to ANTIROBE for the main efficacy criterion.

Comparison of mean pocket depth (PD) in mm between Groups I and II on Day 13 ± 1: PP population (n=113 dogs)

Mean PD (mm)	Group I Bay 14-1877 (n=64)	Group II ANTIROBE (n=61)	p-value
Mean (SD)	3.10 (0.71)	3.30 (0.63)	Student t-test p=0.106218
Median	3.2	3.4	
Min - Max	1.17-4.47	1.97-4.95	

For the PP population, no significant difference was detected between the groups for mean PD on Day 13 ± 1 (p=0.106218).

Title:	Evaluation of the efficacy and safety of Bay 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions
Report ID:	27428
Report Issue Date:	19/04/2004
Study type:	Clinical field study
GCP/GLP	GCP

Study results continued:

Non-inferiority test for mean pocket depth (mm) on Day 13 ± 1: ITT population

Descriptive statistics

PD (mm)	Mean	Standard deviation	Standard error	95% LCL of mean	95% UCL of mean
Group I n=59	3.098588	0.7112148	9.26E-02	2.913244	3.283931
Group II n=64	3.30463	0.6257107	8.51E-02	3.133843	3.475416

T-Test (equal-variance and normality assumptions were met)

Alternative hypothesis	t-value	Prob level	Decision (α=0.05)	Power (α=0.05)	Power (α=0.01)
Difference < 0.165 mm	-2.96329	0.00204**	Reject H_0	0.897979	0.715915

Difference: PD_I - PD_{II}; Significance level: α=0.05; Clinically Significant Difference: θ=5% of PD_{II} or 0.165 mm; ** Statistically significant difference (p<0.01)

For the PP population, the inferiority assumption was rejected at the level of p=0.00204. Therefore, BAY 14-1877 was non-inferior to ANTIROBE for the main efficacy criterion, mean Pocket Depth on Day 13 ± 1.

Comparison of pocket depth for the factors treatment, tooth and study day

ANOVA table

Variable	p-value
Treatment effect	0.118424
Subject effect (case no.)	0.000000***
Tooth effect	0.005116**
Time effect	0.000000***
Tooth and treatment interaction	0.719935
Time and treatment interaction	898446
Tooth and time interaction	0.833502

** Statistically significant difference (p<0.01); *** Statistically significant difference (p<0.001)

A significant tooth effect was detected, showing that the corrected PD was higher for the maxillary right canine (MRC) (3.62 ± 0.06 mm, mean ± SE) than for the maxillary left canine (MLC) (3.37 ± 0.06 mm) on day 13 ± 1. However at the group level, no significant difference was detected between the MLC and MRC by Scheffe's *post hoc* procedure for multiple comparison tests. A significant time effect was detected showing that the corrected mean PD decreased between Day 0 and Day 13 ± 1 in both treatment groups. In addition, Scheffe's *post hoc* procedure for multiple comparison tests confirmed a significant decrease of PD at both target teeth for both treatment groups. Therefore the antimicrobial treatment (BAY 14-1877 or ANTIROBE) induced a significant reduction of PD over the study period at both target teeth for both treatment groups.

Title:	Evaluation of the efficacy and safety of Bay 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions		
Report ID:	27428	Report Issue Date:	19/04/2004
Study type:	Clinical field study	GCP/GLP	GCP

Study results continued:

Comparison of the reduction of pocket depth (PD_0, before treatment, minus PD_{13}, after treatment) between Groups I and II (ITT population)

Mean PD (mm)	Group I Bay 14-1877 (n=64)	Group II ANTIROBE (n=61)	p-value
Mean (SD)	0.41 (0.44)	0.27 (0.30)	Student t-test p=0.036651 * Mann-Whitney U-test # p=0.069758
Median	0.24	0.22	
Min - Max	-0.43-1.78	-0.45 – 1.32	

* Student t-test results were compared to the Mann-Whitney U-test results because the normality assumption was not fully met; * Statistically significant difference ($p<0.05$)

The reduction in PD tended to be higher in Group I (Bay 14-1877) than in Group II (ANTIROBE). The difference was significant when using the Student t-test but was slightly above the significance level when using the Mann-Whitney U-test.

Descriptive statistics

PD (mm)	Count	Mean	Standard deviation	Standard error	95% LCL of mean	95% UCL of mean
Group I	0.4140625	0.4370639	5.46E-02	0.3048872	0.5232378	0.4140625
Group II	0.2721311	0.3044534	3.90E-02	0.194157	0.3501053	0.2721311

T-Test (equal-variance and normality assumptions were met)

Alternative hypothesis	t-value	Prob level	Decision (α=0.05)	Power (α=0.05)	Power (α=0.01)
Difference > -0.20 mm	5.0522	0.00001***	Reject H_0	0.999637	0.996206

Difference: $[(PD_0-PD_{13})$ in group I$]-[(PD_0-PD_{13})$ in group II$]$; Significance level: α=0.05; Clinically significant difference: θ= -0.20 mm; *** Statistically significant difference ($p<0.001$)

The inferiority assumption was rejected at the level of $p<0.00001$. Therefore, BAY 14-1877 was non-inferior to ANTIROBE with regard to reduction of pocket depth between Day 0 and Day 13 ± 1. The corrected mean reduction of pocket depth was 0.307 mm [0.230 - 0.384] in Group I and 0.296 mm [0.223 - 0.368] in Group II. No significant difference was detected between the groups for this parameter ($p=0.839583$ compared to $p=0.069758$ when using uncorrected values).

The ANOVA did not detect any statistically significant difference for any of the 3 factors (tooth, probing site or treatment). In addition, no interaction was detected between any combinations of these three factors. Therefore, the reduction of pocket depth induced by treatment with either BAY 14-1877 or ANTIROBE was similar for both teeth and for all three probing sites.

Mean pocket depth and reduction of pocket depth at each probing site

Summary statistics of PD (mm) and reduction of PD (mm) for tooth, probing site and study day

Tooth	Maxillary Left Carnassial				Maxillary Right Canine			
Probing site	Buccal	Distal	Mesial	Mean	Buccal	Distal	Mesial	Mean
Day 0	3.40±0.75	3.72±0.93	3.35±0.79	3.50±0.85	3.87±0.89	3.58±0.78	3.84±0.96	3.76±0.89
Day 13	3.17±0.70	3.41±0.79	3.10±0.73	3.23±0.75	3.59±0.81	3.34±0.79	3.51±0.10	3.48±0.88
Reduction (D0-D13)	0.23±0.26	0.32±0.50	0.24±0.32	0.28±0.40	0.28±0.40	0.24±0.36	0.34±0.44	0.26±0.38

Title:	Evaluation of the efficacy and safety of Bay 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions		
Report ID:	27428	Report Issue Date:	19/04/2004
Study type:	Clinical field study	GCP/GLP	GCP

Summary statistics of PD (mm) and reduction of PD (mm) for treatment, probing site and study day

Group	Bay 14-1877				ANTIROBE			
Tooth	Maxillary Left Carnassial				Maxillary Left Carnassial			
Probing site	Buccal	Distal	Mesial	Mean*	Buccal	Distal	Mesial	Mean*
Day 0	4.02±0.85	3.52±0.83	3.85±0.98	3.81±0.91	3.72±0.91	3.63±0.74	3.83±0.95	3.73±0.87
Day 13	3.71±0.76	3.31±0.84	3.61±0.99	3.55±0.88	3.48±0.86	3.37±0.75	3.40±1.03	3.41±0.88
Reduction (D0-D13)	0.31±0.40	0.21±0.31	0.24±0.34	0.26±0.35	0.24±0.40	0.26±0.39	0.44±0.51	0.31±0.44

Group	Bay 14-1877				ANTIROBE			
Tooth	Maxillary Right Canine				Maxillary Right Canine			
Probing site	Buccal	Distal	Mesial	Mean*	Buccal	Distal	Mesial	Mean*
Day 0	3.62±0.78	3.88±0.93	3.26±0.88	3.60±0.90	3.19±0.66	3.58±0.93	3.44±0.69	3.41±0.79
Day 13	3.37±0.73	3.53±0.78	3.02±0.81	3.31±0.80	2.97±0.62	3.30±0.80	3.19±0.65	3.16±0.71
Reduction (D0-D13)	0.25±0.25	0.35±0.52	0.24±0.34	0.28±0.39	0.21±0.27	0.28±0.48	0.25±0.29	0.25±0.37

Study results continued:

Comparison of maximum Pocket Depth (mm) between Group I and Group II on Day 13: ITT population

Maximum PD (mm)	Group I Bay 14-1877 (n=64)	Group II ANTIROBE (n=61)	p-value
Mean (SD)	4.13 (1.29)	4.46 (0.95)	Student t-test p=0.105692
Median	4.0	4.2	
Min – Max	1.8-10.7	2.7-6.7	

No significant difference was detected between the groups for maximum PD on Day 13 ± 1.

The lingual PD measurements were excluded from the calculation of mean PD and were analysed separately. No significant difference was detected between the treatment groups for lingual PD on Day 13 ± 1 at both the maxillary left carnassial (p=0.880517) and maxillary right canine (p=0.768283).

Mean bleeding on probing (BOP) score on Day 13 ± 1: ITT population

BOP score	Group I Bay 14-1877 (n=64)	Group II ANTIROBE (n=61)	p-value
Mean (SD)	0.27 (0.39)	0.26 (0.30)	Mann-Whitney U-test p=0.669530
Median	0.125	0.125	
Min – Max	0-2	0-1.25	

Mean bleeding on probing (BOP) score on Day 13 ± 1: PP population

BOP score	Group I Bay 14-1877 (n=59)	Group II ANTIROBE (n=54)	p-value
Mean (SD)	0.29 (0.40)	0.27 (0.32)	Mann-Whitney U-test p=0.878108
Median	0.125	0.125	
Min – Max	0-2	0-1.25	

No statistically significant difference was detected between the groups for BOP score on Day 13 ± 1, regardless of the analysis population used, ITT or PP.

Title: Evaluation of the efficacy and safety of Bay 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions

Report ID: 27428 | **Report Issue Date:** 19/04/2004

Study type: Clinical field study | **GCP/GLP** | GCP

Study results continued:

Reduction of BOP score between Day 0 and Day 13 ± 1: PP population

Study day	Bleeding on Probing Score	Group I BAY 14-1877 n=59	Group II ANTIROBE n=54
Day 0	Mean (SD)	0.79 (0.70)	0.70 (0.59)
Day 13	Mean (SD)	0.29 (0.40)	0.27 (0.32)
p-value		Wilcoxon W –test p=0.000003 ***	Wilcoxon W –test p=0.000011 ***

*** Statistically significant difference (p<0.001).

The BOP score decreased significantly in both groups over the study period.

General condition score (GCS) on Day 13 ± 1: ITT population

BOP score	Group I Bay 14-1877 (n=63*)	Group II ANTIROBE (n=61)	p-value
Mean (SD)	0.13 (0.42)	0.10 (0.44)	Mann-Whitney U-test p=0.546120
Min – Max	0-3	0-3	

* 1 missing data (chewing score for dog QUI05 from group I).

None of the 125 dogs presented a GCS score higher than 3 on Day 13 ± 1 compared to 3 dogs on Day 0. No statistically significant difference was detected between the groups for GCS on Day 13 ± 1.

Reduction of GCS score between Day 0 and Day 13 ± 1: ITT population

Study day	Bleeding on Probing Score	Group I BAY 14-1877 n=59	Group II ANTIROBE n=54
Day 0	Mean (SD)	0.64 (1.36)	0.52 (1.25)
Day 13	Mean (SD)	0.13 (0.42)	0.10 (0.42)
p-value		Wilcoxon W –test p=0.007748 **	Wilcoxon W –test p=0.001836 **

** Statistically significant difference (p<0.01).

The GCS decreased significantly over the study period in both groups.

Bacterio-logical results:

Geometric mean sub-gingival bacterial count and 95% confidence limits

	Day 0		Day 13 ± 1	
Trmt group	Bay 14-1877	ANTIROBE	Bay 14-1877	ANTIROBE
Geom. Mean	6.41E+04	3.77E+04	1.28E+04	3.48E+04
95% LCL	3.00E+04	1.89E+04	6.44E+03	1.72E+04
95% UCL	1.37E+05	7.53E+04	2.53E+04	7.01E+04

LCL: Lower Confidence Limit; UCL: Upper Confidence Limit

A significant time effect was detected (p = 0.004164) showing that the bacterial count decreased significantly between Day 0 and Day 13 ± 1. No treatment effect was detected (p = 0.564843). However, a significant time * treatment interaction was detected (p = 0.009361). This was investigated using Scheffe's *post hoc* procedure.

Scheffe's Multiple-Comparison Test for log_{10} bacterial count

Group	Samples	Mean	Significant different from group
Bay 14-1877, D13 ± 1	58	4.105838	Bay 14-1877, D0
ANTIROBE, D13 ± 1	55	4.541061	-
ANTIROBE, D0	62	4.576462	-
Bay 14-1877, D0	65	4.806878	Bay 14-1877, D13 ± 1

The bacterial count decreased significantly (p < 0.05) between Day 0 and Day 13 ± 1 in Group I (BAY 14-1877). No such decrease was detected in Group II (ANTIROBE) over the same period. Therefore, BAY 14-1877 was effective in reducing the bacterial count after a 7-day antimicrobial therapy in contrast to ANTIROBE. All the strains isolated in both groups on Day 0 were susceptible to both BAY 14-1877 and clindamycin.

Product name: Veraflox Tablets | **Active substance:** Pradofloxacin

Title: Evaluation of the efficacy and safety of Bay 14-1877 tablets in the alleviation of clinical signs associated with periodontal disease in dogs under field conditions

Report ID: 27428 | **Report Issue Date:** 19/04/2004

Study type: Clinical field study | **GCP/GLP** | GCP

Investigator's assessment:

Investigator's assessment of product efficacy

Efficacy assessment	Group I Bay 14-1877 (n=64)		Group II ANTIROBE (n=61)		p-value
	No. dogs	%	No. dogs	%	
Very good	9	14.06	6	9.84	Mann Whitney U-test p=0.520162
Good	41	64.06	40	65.57	
Poor	14	21.88	15	24.59	
Very poor	0	0	0	0	
Total	64	100.00	61	100.00	

No statistically significant difference was detected between the groups for the investigator's assessment of product efficacy on Day 13 ± 1.

Investigator's assessment of product acceptability

Efficacy assessment	Group I Bay 14-1877 (n=64)		Group II ANTIROBE (n=61)		p-value
	No. dogs	%	No. dogs	%	
Very good	31	48.40	26	42.60	Mann Whitney U-test p=0.816836
Good	28	43.80	34	55.70	
Poor	3	4.70	1	1.60	
Very poor	2	3.10	0	0.00	
Total	64	100.00	61	99.90*	

* The difference from 100% is due to rounding

No statistically significant difference was detected between the groups for the investigator's assessment of product acceptability on Day 13 ± 1.

Safety results: Two serious adverse events (SAEs) were observed during the study but both of them were classified as unlikely to be product related according to EMEA guidelines. The following adverse events (AEs) were observed in Group I, each in one dog only: diarrhoea, vomiting, sleepiness and otitis. Two dogs in Group II vomited over the treatment period and two others showed diarrhoea.

Conclusions: BAY 14-1877 proved to be safe and effective in the alleviation of clinical signs associated with periodontal disease in dogs. The clinical efficacy of BAY 14-1877 was non-inferior to that of ANTIROBE, a veterinary drug registered in the EU for this indication. BAY 14-1877 was more effective than ANTIROBE in reducing the sub-gingival bacterial count.